

8-2014

ANTIOXIDANT EFFECT OF PEACH SKIN EXTRACTS FROM 13 VARIETIES OF SOUTH CAROLINA GROWN PEACHES

Yueyuan Zhang

Clemson University, zhyueyuan@gmail.com

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses



Part of the [Food Science Commons](#)

Recommended Citation

Zhang, Yueyuan, "ANTIOXIDANT EFFECT OF PEACH SKIN EXTRACTS FROM 13 VARIETIES OF SOUTH CAROLINA GROWN PEACHES" (2014). *All Theses*. 1868.

https://tigerprints.clemson.edu/all_theses/1868

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.

ANTIOXIDANT EFFECT OF PEACH SKIN EXTRACTS FROM 13 VARIETIES OF SOUTH
CAROLINA GROWN PEACHES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Food, Nutrition and Culinary Science

by
Yueyuan Zhang
August 2014

Accepted by:
Dr. Paul Dawson, Committee Chair
Dr. Julie Northcutt
Dr. Ronald Thomas

ABSTRACT

South Carolina is the second largest peach producing state in the United States. Every year, large quantities of peaches do not meet the fresh peach standard and are discarded or used for further processing. The waste stream from discarded and processed peaches includes the skin and this could be recovered as a rich source of antioxidants. The objective of this research was to determine the antioxidant (phenolic) content and antioxidant capacity of peach skin from various peach varieties grown in South Carolina. Chapter 1 is a literature review which covers topics of oxidation in foods, mechanism of lipid oxidation, antioxidants in food processing, peach antioxidants, mechanism of antioxidants *in vivo*, extraction methods and antioxidant analysis. In Chapter 2, color analysis of peach skin from 13 varieties of peaches grown in South Carolina, phenolic content and antioxidant activity of 13 varieties of peach skin were determined. Norman, Cary Mac, Ruby Prince and Flame Prince varieties differed in color compared to other varieties of peaches evaluated. Peach skin extracts were evaluated for total phenolics (TP) assay, DPPH(2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging (DPPH) assay, ferric reducing antioxidant power (FRAP) assay and ferrous ion chelating (FIC) assay. The range of total phenolics content expressed in gallic acid equivalent (GAE mg/g dry weight) was 8.38 – 18.81 for all peach varieties. Top three peach varieties with skins having the greatest antioxidant power were Red Globe, Scarlet Prince, and O'Henry.

ACKNOWLEDGMENTS

I would like to thank all of my professors and friends who helped me along the way.

Throughout two years of life in Clemson, my advisor Dr. Paul Dawson give me continue support in study and research. I would like to express deepest appreciation to him for his patience, great advices and immense knowledge.

Besides my advisor, I would like to express my sincere thanks to my committee member Dr. Julie Northcutt who helped me a lot at the beginning of the experiment and Dr. Ronald Thomas who helped me get solid knowledge of food chemistry.

I also would like to give special thanks to Dr. Inyee Han who is enthusiastic and always there helping me get out of tough time and Dr. James Rieck who dedicate a lot of time helping me with data analysis. I also would like to thank Mac Marsh for his generous help with peach sampling. Without their help, I can never complete this study successfully.

Last but not least, I would like to thank my parents Xiaoxia Yue, Gensheng Zhang, my sister Yuetuan Zhang and my boyfriend Jingyuan Yan for their support and encouragement all these years.

Table of Contents

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vi
CHAPTER ONE: REVIEW OF OXIDATION AND ANTIOXIDANT	1
Introduction	1
Oxidation in Foods	1
Lipid Oxidation Mechanism	2
Antioxidants in Food Processing	4
South Carolina Peach Varieties	7
Antioxidants in Peaches	8
Mechanism of antioxidant action in vitro	9
Methods to Extract Antioxidants from Plant Tissue	11
Antioxidant Analysis	14
References	18
CHAPTER TWO: ANTIOXIDANT ACTIVITY OF 13 VARIETIES OF PEACH SKINS	28
Abstract	28

Table of Contents (Continued)

	Page
Introduction	28
Material and Methods	30
Results and Discussion	35
Conclusion	47
References	50
APPENDICES	53
Appendix A: 13 Varieties of Peach Extracts	54
Appendix B: 13 Varieties of Peach Samples	55
Appendix C: Description of 13 cultivars of peaches	58
Appendix D: Antioxidant activity of 13 varieties of peach skins	60
Appendix E: Correlation of four antioxidant assays	62

LIST OF TABLES

	Page
Table 1. Color analysis of 13 cultivars of peach skins.....	38
Table 2. Correlation of four antioxidant assays.....	46
Table 3. Correlation of color and total phenolic content	47

LIST OF FIGURES

	Page
Figure 1. Mechanism of autoxidation.....	4
Figure 2. Chemical structures of synthetic antioxidants	5
Figure 3. Chemical structures of natural antioxidants	7
Figure 4. Examples of antioxidant in peaches	9
Figure 5. Mechanism of antioxidant action <i>in vitro</i>	11
Figure 6. Color Analysis (L*) of 13 cultivars of peaches	36
Figure 7. Color Analysis (C*) of 13 cultivars of peaches	37
Figure 8. Color Analysis (Hue) of 13 varieties of peaches	37
Figure 9. Antioxidant activity of Total Phenolics assay	40
Figure 10. Box and whisker of Total Phenolics assay (Dry Weight).....	40
Figure 11. Antioxidant activity of DPPH assay.....	42
Figure 12. Box and whisker plot of DPPH assay	42
Figure 13. Antioxidant activity of FRAP assay	43
Figure 14. Box and whisker plot of FRAP assay	44
Figure 15. Antioxidant activity of FIC assay.....	45
Figure 16. Box and whisker plot of FIC assay	45

CHAPTER ONE: REVIEW OF OXIDATION AND ANTIOXIDANT

Introduction

Lipid oxidation leads to the development of undesirable flavors and odors of food products and has been a major concern of the food industry. Currently, synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butyl hydroquinone (TBHQ) have been widely used to retard lipid oxidation in foods. However, there are consumer concerns about the use of synthetic compounds as food additives (Wichi, 1988; Grice, 1986). Therefore, the demand for natural antioxidants is increasing. In 2012, the US peach total production reached 978,260 tons with utilized production reaching 965,420 tons (USDA, 2013). South Carolina is the second largest peach producing state in the United States. In 2012, South Carolina had a total of 17,000 acres of peach trees with total peach production reached 75,000 tons which was valued over 74 million dollars. However, in 2012 unharvested peaches totaled 3,700 tons, ranking first of all the states. Harvested but not sold peaches also reached 1,050 tons, ranking second of all states (USDA, 2013). Peaches are a good source of natural antioxidants and there is a potential for recovery of lost antioxidants from those unutilized peaches.

Oxidation in Foods

Lipids are the primary components of many foods and can be related to the development of product flavor, texture and color. However, lipids can be easily

degraded by oxygen, leading to a chain of chemical reactions resulting in the formation of undesirable flavors and off odors (Gray et al., 1996). Metals, light, temperature and enzymes are factors that can accelerate the oxidation process (Shahidi, 1997). Foods with high levels of unsaturated fats such as meat products, dairy products, fish and oils are especially sensitive to lipid oxidation.

Secondary products of lipid oxidation such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HN) can interact with proteins and amino acids (Shahidi, 1997). Those interactions can also lead to undesirable color and textural changes of foods. Lipid oxidation in particular, has been a major concern of the food industry. Oxidative deterioration of lipids renders the product unacceptable to consumer.

Lipid Oxidation Mechanism

There are three general mechanisms of lipid oxidation, namely autoxidation, photosensitized oxidation and enzyme catalyzed oxidation. Since autoxidation cannot be controlled by blanching (enzyme inactivation) or exclusion of light energy and is a common reaction leading to oxidative deterioration, it is of importance to the food industry. The three phases of autoxidation are initiation, propagation and termination (Figure 1).

Initiation

Oxidation is initiated when free radicals are formed via hydrogen atom abstraction by oxidizing agents such as transition metals, singlet oxygen and other free radicals. During

this process, lipid free radicals (L^\bullet) are generated and rapidly react with molecular oxygen leading to the formation of the lipid peroxy radical (LOO^\bullet). (Frankel, 1984; Pokorny et al., 2001)

Propagation

After initiation, a chain reaction accelerates oxidation via propagation. The peroxy radical abstracts a hydrogen atom which can originate from another unsaturated fatty acid, forming a lipid hydroperoxide ($LOOH$) and another L^\bullet . Hydroperoxide is a highly unstable primary product of oxidation. It can be degraded into secondary products such as aldehydes, ketones, acids and alcohols which create off-odors and flavors. (Frankel, 1984; Pokorny et al., 2001)

Termination

The progression to termination reactions starts when free radicals begin to bind to one another to form more stable, nonradical species. At this point, one cycle of lipid oxidation is completed. However, there can be reinitiation causing the cycle to repeat (Frankel, 1984; Pokorny et al., 2001).

Initiation: $\text{LH} \rightarrow \text{L}\bullet + \text{H}\bullet$

Propagation: $\text{L}\bullet + \text{O}_2 \rightarrow \text{LOO}\bullet$

$\text{LOO}\bullet + \text{LH} \rightarrow \text{LOOH} + \text{L}\bullet$

Termination: $\text{LOO}\bullet + \text{LOO}\bullet \rightarrow \text{LOOL} + \text{O}_2$

$\text{LOO}\bullet + \text{L}\bullet \rightarrow \text{LOOL}$

$\text{L}\bullet + \text{L}\bullet \rightarrow \text{LL}$

Figure 1. Mechanism of autoxidation

Antioxidants in Food Processing

Antioxidants are added to food products to retard lipid oxidation. Food antioxidants are defined as “substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation” by the Food and Drug Administration (FDA, 2013).

Based on the mechanism of antioxidant action in vitro, antioxidants can be classified into two groups, namely primary antioxidants and secondary antioxidants. Antioxidants that react with lipid radicals to create more stable products are primary antioxidants while others are categorized as secondary antioxidants. (Pokorny et. al., 2001)

Another common antioxidant classification is based on the source (natural or synthetic) of antioxidants.

Synthetic antioxidants

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) have been used by the food industry for many years (Figure 2). Compared to natural antioxidants, synthetic antioxidants have proven effectiveness in a variety of food systems at a relatively low cost. However, the controversy about the safety of those compounds continues. It has been reported that addition of BHA to the diet of rats induced high incidences of papilloma and squamous cell carcinoma of the forestomach (Ito et al., 1985). Also, it has been observed that BHT had specific toxic effects on lungs and induced liver tumors in long-term animal experiments (Kahl and Kappus, 1993). The inducement of animal tumors in the forestomach by BHA was dose dependent (Kahl and Kappus, 1993). Conversely, another study indicated that there was no significant association with stomach cancer risk with typical intake of BHA and BHT (Botterweck et al., 2000). Williams et al. (1999) also pointed out that BHA and BHT pose no cancer hazard and may be anticarcinogenic at current levels of food additive use.

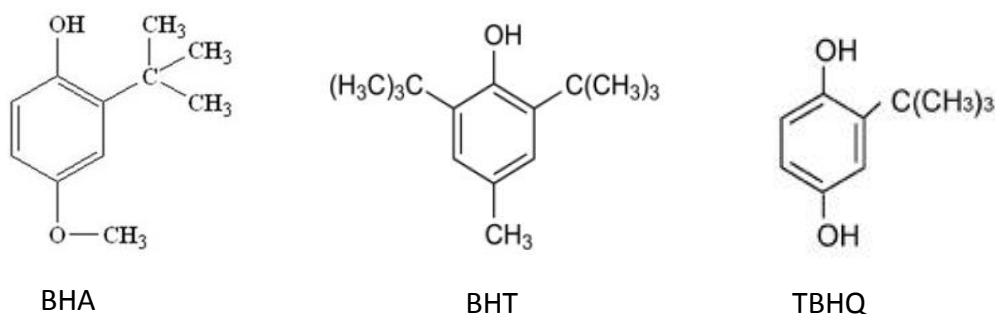


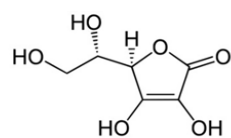
Figure 2. Chemical structures of synthetic antioxidants

Natural antioxidants

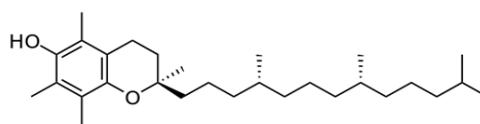
Natural antioxidants such as ascorbic acid, anthocyanins, tocopherols, catechins and carotenoids (Figure 3) are widely distributed in plants including fruits, vegetables, nuts, seeds, flowers and bark. They have been reported to be more potent, efficient and safer than synthetic antioxidants (Pokorny et. al., 2001). For instance, natural 2R,4'R,8'R- α -tocopherol is more active than synthetic racemic α -tocopherol primarily because α -tocopherol transfer protein selectively recognizes natural α -tocopherol (Hudson, 1990).

Natural antioxidants are not only effective in reducing lipid oxidation in food products, but also may contribute to reducing the incidence of cardiovascular diseases, cancers, osteoporosis, neurodegenerative diseases and diabetes mellitus (Scalbert et al., 2005). Additional studies show that polyphenols also possess significant anti-inflammatory, antioxidant, anti-aging (Pandey and Rizvi, 2009) and anti-DNA damaging effects (Nichols and Katiyar, 2010).

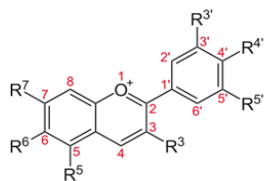
Many studies have focused on finding sources of natural antioxidants that can be used in place of synthetic antioxidants. Sources have included rosemary (Aruoma et al., 1992), sage (Djarmati et al., 1991), Jabuticaba skin (Santos et al., 2010), grape (Nawaz et al., 2006), apple (Virot et al. 2010), orange peel (Khan et al., 2010) and Sparganii rhizome (Wang et al. 2013). Natural antioxidants such as tocopherols and extracts from rosemary or sage have been proven effective and are being used in food systems.



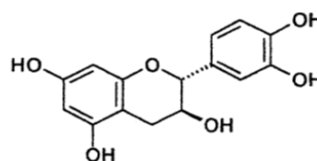
Ascorbic acid



α -Tocopherols



Anthocyanin



Catechin

Figure 3. Chemical structures of natural antioxidants

South Carolina Peach Varieties

Peaches are one of the most popular fruits worldwide partly due to its good taste and high nutritional value. In the early 1970s, the annual peach consumption per capita in the U.S. reached a peak of 13 pounds. By 2008, the annual consumption had dropped to 8.8 pounds per person. (Brunke et al., 2013) Peaches contain carbohydrates, organic acids, proteins, lipids, pigments, phenolic compounds, volatile compounds, vitamins and minerals which contribute to their nutritional value. Peaches can be categorized as clingstone, freestone or semi-freestone, depending on the degree the flesh adheres to the stone. Peaches can also be categorized based their flesh color. Different cultivars give various peach sizes, color, taste and nutritional value. (Larue and Johnson, 1989)

The history of planting peach trees dates back to 1000 B.C. Today, the peach, which is native to China, has become one of the most popular fruits grown throughout the

world's north and south temperate zones. Hundreds of different peach cultivars have been developed with different countries having their own preferred cultivars.

In 2012, the US peach total production reached 978,260 tons with the top three peach producing states being California, South Carolina and Georgia. South Carolina is the second largest peach producing state in the United States. By 2012, South Carolina had a total of 17,000 acres of peach trees with total peach production of 75,000 tons and a value of over 74 million dollars. (USDA, 2013).

The peach harvest season in SC runs from May through September and these fresh peaches are highly perishable. Since the varieties grown in SC are suited primarily for the fresh market, it is recommended that peaches should be consumed in two weeks.

From 2010 to 2012, thousand tons of peaches were discarded in South Carolina. In 2012, unharvested peaches reached 3,700 tons, ranking first of all the states. Harvested but not sold peaches also reached 1,050 tons, ranking second among all states. (USDA, 2013) The possibility of extracting antioxidants from the peach skin waste stream has not been reported.

Antioxidants in Peaches

It has been reported that phenolic compounds play an important role in antioxidant activity of peaches (Tomás-Barberán et al., 2001). Major phenolic compounds in peaches are hydroxycinnamates, procyanidins, flavonols and anthocyanins. Other antioxidants known to be present in peaches include ascorbic acid and carotenoids.

However, the content of ascorbic acid and carotenoids in peaches are relatively low (Tomás-Barberán et al., 2001). Since phenolic compounds are concentrated in peach skin, the skin is a potential source for recovering antioxidants. (Layne, 2008)

Phenolic antioxidants belong to a class of chemical compounds containing a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon. Examples of phenolic compounds are caffeic acid, chlorogenic acid, leucoanthocyanins, catechins and flavonols (Figure 4). With a few exceptions, phenols in foods have been shown to be more effective at preventing lipid peroxidation than many vitamins (Rice-Evans et al. 1997). Phenolic compounds can be classified as simple phenols or polyphenols based on the number of phenol units in a molecule. In most cases, polyphenols exhibit greater antioxidant activity than monophenols (Amarowicz et al., 2000).

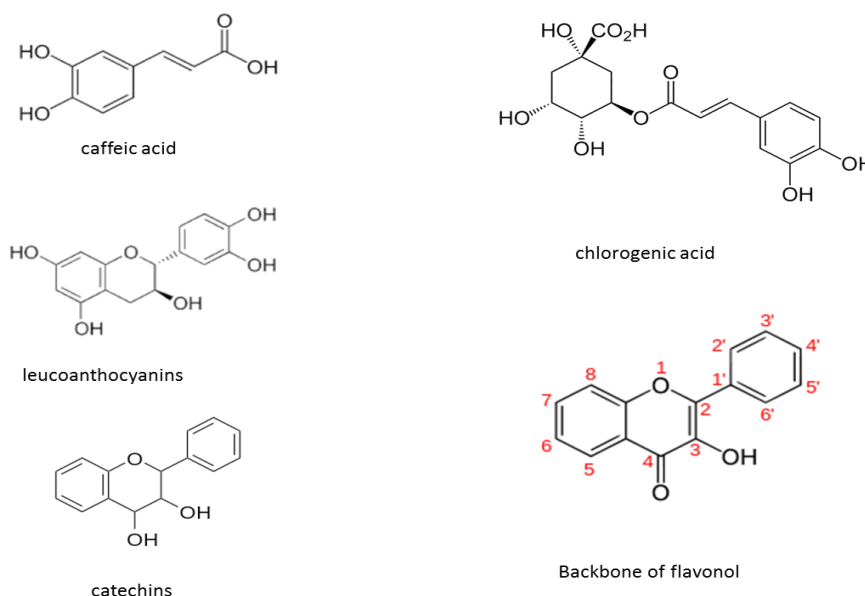


Figure 4. Examples of antioxidant in peaches

Mechanism of antioxidant action in vitro

To retard lipid oxidation, antioxidants can function by either inhibiting the formation of free alkyl radicals in the initiation step or interrupting the propagation of the free radical chain (Ingold, 1968). In other words, antioxidants can both delay the initiation or slow propagation of lipid oxidation. Free radical formation can be delayed by the use of metal chelating agents, singlet oxygen inhibitors, and peroxide stabilizers. The propagation of free radicals can be slowed by the donation of hydrogen from the antioxidants and by restriction of metal ions by metal chelating agents.

Primary antioxidants interfere with lipid autoxidation by rapid donation of hydrogen atoms to lipid radicals according to reactions (a), (b) or (c) shown in Figure 5. Alternative mechanisms only become important under special conditions such as very low oxygen pressures, very high concentrations of antioxidant or very low rates of chain initiation (Hudson, 1990).

In contrast, secondary antioxidants can reduce the rate of chain initiation by a variety of mechanisms including scavenging oxygen, binding metal ions, decomposing hydroperoxides to non-radical species, absorbing UV radiation and deactivating singlet oxygen. When operating as an oxygen scavenger, antioxidants such as ascorbic acid are oxidized via reaction (d) in Figure 5. Some antioxidants such as β -carotene can retard lipid oxidation by quenching of singlet oxygen as shown in reaction (e). Metal ions in food products often act as pro-oxidants by electron transfer, liberating radicals from fatty acids or hydroperoxides via reactions (f), (g) and (h) (Figure 5). Chelating agents

can react with metal ions by forming σ -bonds thus reducing the pro-oxidative effect of metal ions and increasing activation energy of initiation reactions considerably.

(Hudson, 1990)

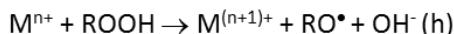
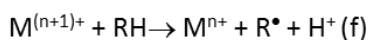
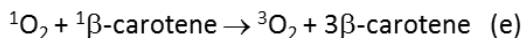
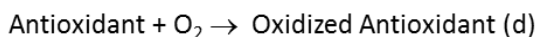
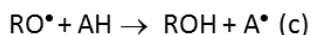
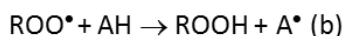
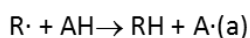


Figure 5. Mechanism of antioxidant action *in vitro*

Methods to Extract Antioxidants from Plant Tissue

Traditional extraction methods of phenolic antioxidants from plant tissue include soxhlet, solid-liquid and liquid-liquid extraction. Disadvantages of those methods are time intensity, high solvent consumption and high risk of thermal degradation of target compounds. Alternative extraction methods include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurised solvent extraction (PSE). Kaufman and Christen (2002) pointed out that MAE and PSE can reduce both solvent consumption and extraction times. At the same time, the extraction yields of the analytes are equivalent to or even higher than those

obtained with conventional methods (Kaufmann and Christen, 2002). Since UAE and MAE are relatively simple and cost low, many researchers have adopted these methods.

Microwave assisted extraction (MAE)

Microwaves are electromagnetic radiations with frequencies ranging from 0.3 to 300 GHz (Camel, 2001). Owing to their electromagnetic nature, microwaves possess electric and magnetic fields which are perpendicular to each other. Two mechanisms of microwave heating were proposed, namely, dipolar rotation and ionic conduction.

Dipole rotation is based on the fact that many molecules exist as electric dipoles. When placed in an electromagnetic field, dipoles attempt to align themselves according to the polarity of the field, changing about 4.9×10^9 times per second (Onuska and Terry, 1995). The constant rotation of the molecules results in heating which is very fast and simultaneous throughout sample. Ionic polarization can also be induced by electric field in solution. Researchers indicated that the medium resists ionic currents which are formed in ionic polarization causing friction within the medium and therefore heat is liberated by the Joule effect. Ionic polarization depends on the size and charge of the ions present in the solution. (Kaufmann and Christen, 2002)

MAE of compounds has been reported for various samples since 1985. Extraction of natural products has included essential oil, carotenoids, steroids, taxanes (Kaufmann and Christen, 2002). Singh et al. (2011) optimized the MAE of phenolic antioxidants from potato. Hao et al. (2002) reported the possibility of MAE of artemisinin from *Artemisia*

annua. L. Pan et al. (2000) applied MAE on glycyrrhizic acid from licorice root. MAE is the process of using microwave energy to heat solvents while in contact with a sample and thereby extracting compounds from the sample into the solvent. This approach of microwave heating usually allows for a reduction of solvent volume needed for extraction.

Ultrasound assisted extraction (UAE)

Ultrasound is high frequency (>20 kHz) sound wave pressure which is greater than the upper limit of human hearing range. UAE works by passing ultrasonic energy in the form of waves into a sample during extraction. It generates alternating low-pressure and high-pressure waves in liquids constantly, leading to the formation and violent collapse of small vacuum bubbles. Bubbles grow during the rarefying phase of the sound wave and collapse during the compression phase. On collapse, ultrasound energy converts into mechanical energy in the form of shock waves which are equivalent to several thousand atmospheres of pressure. The whole process of bubble nucleation, growth and collapse is known as cavitation (Júnior et al., 2006). It is believed that the rapid increases of pressure and temperature caused by cavitation are responsible for the disruption of cellular membranes, thus improving efficiency and accelerating extraction (Soria and Villamiel, 2010).

Ultrasound-assistant extraction has been used for extracting various compounds from food materials. Compared with other methods such as SFE, it has the advantage of low

cost and minimal instrumental requirements. An ultrasonic probe system or an ultrasonic bath maybe used to perform UAE. Numerous reports applying this method include herbal and oil extraction (Vinatoru, 2001), protein extraction (Moulton and Wang, 1982), polyphenol extraction (Khan et al., 2010; Wang et al., 2013), anthocyanins (Cai et al., 2003), tartaric acid (Palma and Barroso, 2002), aroma compounds (Vila et al., 1999), polysaccharides and functional compounds (Sun et al., 2004).

Antioxidant Analysis

Since 1958, numerous *in vitro* antioxidant assays have been proposed including DPPH free radical scavenging assay (Kurechi et al., 1980), ferrous chelating capacity (FIC) (Decker and Welch, 1990), trolox equivalent antioxidant capacity assay (TEAC) (Miller et al., 1993), oxygen radical absorbing capacity (ORAC) (Cao et al., 1993), total radical trapping antioxidant parameter (TRAP) (Lissi et al., 1995), ferric ion reducing antioxidant power assay (FRAP) (Benzie and Strain, 1996) and ABTS assay (Re et al., 1999). All of these antioxidant assays have been applied to test the antioxidant activity in vitro. The assays have primarily been used for fruits, vegetables, other plant extracts, beverages and nutritional supplements. These assays can be classified into three types: hydrogen atom transfer (HAT) assay, electron transfer (ET) assay and other assays (Huang et al., 2005). Considering convenience and other limitations, total phenolic assay, DPPH assay and FRAP assay are the most popular methods applied. As antioxidants act by several

mechanisms and no one assay can capture the different modes of action of antioxidant, several different methods should be used in evaluation.

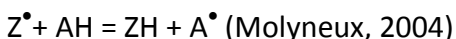
Total Phenolic assay

Total phenolic content can be determined by the Folin-Ciocalteu colorimetry (FC) method. It has the advantage of a fairly equivalent response to different phenols, with the disadvantage of responding to sulfur dioxide and sugar. It has been used extensively with many types of food including wine, whiskies, fruit juices and plant tissues. In this method, the FC reagent oxidizes phenol compounds to phenolate (phenol ions) in the sample, while the FC reagent is reduced (gain ions from phenol) to produce blue molybdenum-tungsten complex. The phenols are oxidized rapidly in alkaline conditions to give appreciable concentrations of phenolate ions. However, acidic FC reagent and the blue complex formed are unstable in alkaline conditions. Therefore, the moderate pH of around 9-10 is used to achieve reasonably rapid production and relatively long retention of maximum color. Also, excess amount of FC reagent is used so that enough FC reagent will survive the alkaline condition long enough to react with all the phenolate. Gallic acid is often used as a standard. Results are often expressed in mg gallic acid equivalents (GAE) per liter (Singleton et al., 1999).

DPPH free radical scavenging assay

DPPH free radical scavenging method determines the free radical scavenging capacity of a compound. DPPH is one of a few stable and commercially available organic nitrogen

radicals which have UV-absorbance at 517nm. When reacted with an antioxidant which can donate hydrogen atoms, the reduced form of DPPH is generated, accompanied by the disappearance of the violet color. (Kurechi et al., 1980) Representing the DPPH radical by Z^\bullet and the donor molecule by AH, the reaction can be expressed as:



where ZH is the reduced form and A^\bullet is free radical produced.

The scavenging activity can be calculated as follows or expressed as ascorbic acid equivalent.

$$\text{DPPH scavenging activity} = [(A_0 - A_1)/A_0] \times 100\% \text{ (Ardestani et al., 2007)}$$

A_0 is absorbance of a control lacking any radical scavenger

A_1 is absorbance of the remaining DPPH in the presence of scavenger

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. The first step of this assay is mixing FeCl_3 , acid buffer, and TPTZ reagent (10 mM 2,4,6-tripyridyl-s-triazine in 40 mM HCl) to form the oxidant, $\text{Fe}^{\text{III}}(\text{TPTZ})_2\text{Cl}_3$ which has a pale yellow color. Then, when an antioxidant is added, the $\text{Fe}(\text{TPTZ})_2\text{Cl}_3$ is reduced to $\text{Fe}^{\text{II}}(\text{TPTZ})_2\text{Cl}_2$, which gives a very intense navy blue color (Benzie and Strain, 1996). The reaction is nonspecific, and any half-reaction which has a less-positive redox potential, under

reaction conditions, than the $\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}$ -TPTZ half reaction will drive Fe^{III} -TPTZ reduction. Test conditions favor reduction of the complex and excess Fe^{III} should be used, thereby, color development will correlate with the reducing ability of the antioxidant. The absorbance can be measured spectrophotometrically at 593 nm to reflect the amount of iron reduced and correlated with antioxidant activity. (Muller et al., 2011)

Ferrous ion chelating (FIC) assay

FIC (Ferrous ion chelating activity) assay depends on chelating metal ions. When FeCl_2 and antioxidant are mixed, they can bind together to form an antioxidant- Fe^{2+} compound. After that, Ferrozine is added to react with the remaining FeCl_2 , forming a Ferrozine- Fe^{2+} compound which gives a violet color that can be detected spectrophotometrically at 562nm. Ferrous ion chelating ability can be calculated as follows.

$$\text{Chelating ability} = [(A_0 - A_1)/A_0] \times 100\%,$$

A_0 is absorbance of a control lacking any antioxidant,

A_1 is absorbance of the remaining Ferrozine- Fe^{2+} in the presence of antioxidant. (Decker and Welch, 1990; Premysl et al., 2011; Bena-Marie et al., 2010)

References

Amarowicz, R.; Naczek, M.; Shahidi, F. Antioxidant activity of crude tannins of Canola and Rapeseed Hulls. *JAOCS*. 2000, 77(9).

Aruoma, O.I.; Halliwell, B.; Aeschbach, R.; Löliger, J. Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid, *Xenobiotica*. 1992, Feb. 22(2), 257-68.

Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem*. 1996, 239, 70-76.

Botterweck, A.A.; Verhagen, H.; Goldbohm, R.A.; Kleinjans, J.; Van den Brandt P.A. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study. *Food Chem. Toxicol*. 2000, 38(7), 599-605.

Cai, J.; Liu, X.; Li, Z.; An, C. Study on extraction technology of strawberry pigments and its physicochemical properties. *Food and Fermentation Industries*. 2003, 29, 69–73.

Camel, V. Recent extraction techniques for solid matrices—supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst*. 2001, 126, 1182-1193.

Cao, G.; Alessio, H.M.; Cutler, R.G. Oxygen-radical absorbance capacity assay for antioxidants. *Free radical biology and medicine*. 1993, 14(3), 303-311.

Decker, E. A.; Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 1990, 38, 674–677.

Djarmati, Z.; Jankov, R. M.; Schwirtlich, E.; Djulinac, B.; Djordjevic, A. High antioxidant activity of extracts obtained from sage by supercritical CO₂ extraction. *Journal of the American Oil Chemists Society*. October 1991, 68(10), 731-734.

FDA (U.S. Food and Drug Administration). Code of Federal Regulations Title 21, Chapter 1 Food and Drug Department of Health and human services, Subchapter B – Food for human consumption, Part 170. 2013, CFR170.3.

Frankel, E. N. Lipid oxidation: Mechanisms, products and biological significance. *J. Am. Oil Chem. Soc.* 1984, 61(12), 1908-1917.

Graya, J.I.; Gomaa, E.A.; Buckley, D.J. Oxidative quality and shelf life of meats. *Meat Science*. 1996, 43(1), 111–123.

Grice, H.C. Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. *Food and Chemical Toxicology*. 1986, 24, 1127–1130.

Hao, J.; Han, W.; Huang, S.; Xue, B.; Deng, X. Microwave-assisted extraction of artemisinin from *Artemisia annua* L. *Separation and Purification Technology*. 2002, 28(3), 191–196.

Huang, D.; Qu, B.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* 2005, 53, 1841-1856.

Hudson, B.J.F. Food antioxidants. In *The Mechanism of Antioxidant Action in Vitro*; Gordon, M.H.; Elsevier Science Publishers; 1990; 4-14.

Hudson, B.J.F. Food antioxidants. In *Natural Antioxidants Exploited Commercially*; Schuler, P.; Elsevier Science Publishers; 1990; 104.

Ingold, K.U. Inhibition of autoxidation. *Adv. Chem. Ser.* 1968, 75, 296-305.

Ito, N.; Fukushima, S.; Tsuda, H. Carcinogenicity and Modification of the Carcinogenic Response by BHA, BHT, and Other Antioxidants. *Crit Rev Toxicol.* 1985, Vol. 15, No. 2, Pages 109-150.

Júnior, D.S.; Krug, F.J.; de Godoi Pereira, M.; Korn, M. Currents on ultrasound-assisted extraction for sample preparation and spectroscopic analytes determination. *Applied Spectroscopy Reviews*. 2006, 41, 305.

Kahl, R; Kappus, H. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E, *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*. 1993, 196(4), 329-338.

Kaufmann, B.; Christen, P. Recent Extraction Techniques for Natural Products: Microwave-assisted Extraction and Pressurised Solvent Extraction. *Phytochem. Anal.* 2002, 13, 105–113.

Khan, M. K.; Abert-Vian, M.; Fabiano-Tixier, A.; Dangles, O.; Chemat, F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chem.* 2010, 119, 851–858.

Kurechi, T.; Kikugawa, K.; Kato, T. Studies on the Antioxidants Hydrogen Donating Capability of Antioxidants to 2,2-Diphenyl-1-picrylhydrazyl. *Chem. Pharm. Bull.* 1980, 28(7), 2089-2093.

Larue, J.; Johnson, S. Peaches, plums, and nectarines growing and handling for fresh market. Publications University of California. 1989.

Layne, D.R.; Bassi, D. The peach botany, production and uses. 2008. CAB international.

Lissi, E.; Salim-Hanna, M.; Pascual, C.; del Castillo, M.D. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced

chemiluminescence measurements. *Free Radical Biology and Medicine*. 1995, 18(2), 153-158.

Liu, J.; Wang, C.; Wang, Z.; Zhang, C.; Lu, S.; Liu, J. The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chem*. 2011, 126(1), 261-269.

Lue, B.; Nielsen, N.S.; Jacobsen, C.; Hellgren, L.; Guo, Z.; Xu, X. Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays. *Food Chem*. 2010, 123(2), 221-230.

Miller, N.J.; Rice-Evans, C.A.; Davies, M.J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. *Clin. Sci*. 1993, 84, 407-412.

Mladenka, P.; Macakova, K.; Fillipsky, T.; Zatloukalova, L.; Jahodar, L.; Bovicelli, P.; Silvestri, L.P.; Hrdina, R.; Saso, L. In vitro analysis of iron chelating activity of flavonoids. 2011, 105(5), 693-701.

Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*. 2004, 26(2), 211-219.

Moulton, J.; Wang, C. A pilot plant study of continuous ultrasonic extraction of soybean protein. *Journal of Food Science*, 1982, 47, 1127–1129.

Muller, L.; Frohlich, K.; Bohm, V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (α TEAC), DPPH assay and peroxy radical scavenging assay. *Food Chem.* 2011, 129 (1), 139-148.

Nawaz, H.; Shi, J.; Mittal, G.S.; Kakudac, Y. Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Separation and Purification Technology.* 2006, 48, 176–181.

Nichols, J.A.; Katiyar, S.K. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch Dermatol Res.* 2010, Mar, 302(2), 71-83.

Onuska, F.I.; Terry K.A. Microwave extraction in analytical chemistry of pollutants: polychlorinated biphenyls. *J High-Resol Chromatogr.* 1995, 18, 417-421.

Palma, M.; Barroso, G. Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products. *Analalytica Chemica Acta.* 2002, 458, 119–130.

Pan, X.; Liu, H.; Jia, G.; Shu, Y.Y. Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochemical Engineering Journal.* 2000, 5(3), 173–177.

Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev.* 2009, 2(5), 270–278.

Pokorny, J.; Yanishlieva, N.; Gordon, M. Antioxidants in Food: Practical Application. In The development of oxidative rancidity in foods, Gordon, M. 2001, pp 11 Woodhead Publishing Limited, Florida, USA.

Pokorny, J.; Yanishlieva, N.; Gordon, M. Antioxidants in Food: Practical Application. In The development of oxidative rancidity in foods, Gordon, M. 2001, pp 17 Woodhead Publishing Limited, Florida, USA.

Pokorny, J.; Yanishlieva, N.; Gordon, M.H. Antioxidant in Food: Practical Application. In Introducing Natural Antioxidant. Shi, H.; Noguchi, N.; Niki, E. 2001, pp 147. Woodhead Publishing Limited, Florida, USA.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 1999, 26(9/10), 1231-1237.

Rice-Evans, C.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. Trends in plant science. 1997, 2(4), 152–159.

Santos, D.T.; Veggi, P.C.; Angela, M.; Meireles, A. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation. Journal of Food Engineering. 2010, 101, 23–31.

Scalbert, A.; Manach, C.; Morand, C.; Rémésy, C.; Jiménez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr.* 2005, 45(4), 287-306.

Shahidi, F. (Editor). *Natural antioxidants: chemistry, health effects and applications.* 1997. AOCS Press, Champaign, Illinois.

Singh, A.; Sabally, K.; Kubow, S.; Donnelly, D.J.; Garipey, Y.; Orsat, V.; Raghavan, G.S.V. Microwave-Assisted Extraction of Phenolic Antioxidants from Potato Peels. *Molecules.* 2011, 16, 2218-2232.

Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology.* 1999, 299, 152-178.

Soria A.C. and Villamiel M. Effect of ultrasound on the technological properties and bioactivity of food: a review. *Trends in Food Science & Technology.* 2010, 21, 323-331.

Sun, X.; Sun, F.; Zhao, H.; Sun, C. Isolation and characterization of cellulose from sugarcane bagasse. *Polymer Degradation and Stability.* 2004, 84, 331–339.

Tomás-Barberán, F.A.; Gil, M.I.; Cremin, P.; Waterhouse, A.L.; Hess-Pierce, B.; Kader, A.A. HPLC–DAD–ESIMS Analysis of Phenolic Compounds in Nectarines, Peaches, and Plums *J. Agric. Food Chem.* 2001, 49 (10), 4748–4760.

Tuba, A.K.; Gulcin, I. Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*. 2008, 174, 27-37.

USDA. Noncitrus fruits and nuts 2012 preliminary summary. 2013, pp 58.

http://www.nass.usda.gov/Publications/Todays_Reports/reports/ncit0113.pdf

Vila, D.; Mira, H.; Lucena, R.; Fernandez, R. Optimization of an extraction method of aroma compounds in white wine using ultrasound. *Talanta*. 1999, 50, 413–421.

Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*. 2001, 8, 303–313.

Virost, M.; Tomao, V.; Bourvellec, C.L.; Renard, C.M.C.G.; Chemat, F. Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction. *Ultrasonics Sonochemistry*, 2010, 17, 1066–1074.

Wang, X.; Wu, Y.; Chen, G.; Yue, W.; Liang, Q.; Wu, Q. Optimisation of ultrasound assisted extraction of phenolic compounds from *Sparganii* rhizoma with response surface methodology. *Ultrasonics Sonochemistry*. 2013, 20, 846–854.

Wichi, H.P. Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. *Food and Chemical Toxicology*. 1988, 26, 717–723.

Williams, G.M.; Latropoulos, M.J.; Whysner, J. Safety Assessment of Butylated Hydroxyanisole and Butylated Hydroxytoluene as Antioxidant Food Additives. *Food and Chemical Toxicology*. 1999, 37(9–10), 1027–1038.

Wootton-Bread, P.C.; Moran, A.; Ryan, L. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Research International*. 2011, 44(1), 217-224.

Zulueta, A.; Maria, J.; Esteve; Frigola, A. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chem*. 2009, 114 (1), 310-316.

CHAPTER TWO: ANTIOXIDANT ACTIVITY OF 13 VARIETIES OF PEACH SKINS

Abstract

Color analysis, phenolic content and antioxidant activity of peach skin from 13 varieties of peaches grown in South Carolina were determined. Color analysis indicated that Norman, Cary Mac, Ruby Prince and Flame Prince differed from other varieties of peaches. Antioxidant activity of peach skin extracts were evaluated by total phenolics (TP) assay, DPPH free radical scavenging (DPPH) assay, ferric reducing antioxidant power (FRAP) assay and ferrous ion chelating (FIC) assay. Results indicated that the range of total phenolics content was 8.38 – 18.81 (Gallic Acid Equivalent mg/g Dry weight) or 135.92 – 461.14 (Gallic acid equivalent mg/100g fresh weight). The top three peach varieties with skins having the greatest antioxidant capacity were Red Globe, Scarlet Prince, and O'Henry.

Key words

Peach skin; Antioxidant; Total phenolics; DPPH assay; FRAP assay

1. Introduction

Peaches (*Prunus persica*) are one of the most popular fruits worldwide partly due to its good taste and nutrient composition (Block, Patterson, & Subar, 1992; Ness, & Powles, 1997). In the early 1970s, the annual per capita consumption of peaches in the U.S. reached the peak at 13 pounds. By 2008, the annual consumption had dropped to 8.8 pounds per person (Brunke et al., 2013). In 2012, the US peach total production reached 978,260 tons with utilized production reached 965,420 tons(USDA, 2013). South

Carolina is the second largest peach producing state in the United States. By 2012, South Carolina had a total of 17,000 acres of peach trees with total peach production reached 75,000 tons which was valued over 74 million dollars. However, in 2012 unharvested peaches reached 3,700 tons, ranking first of all the states. Harvested but not sold peaches also reached 1,050 tons, ranking second of all states (USDA, 2013). Those unutilized peaches could be a good source of natural antioxidants. The possibility of extracting antioxidant from South Carolina peach skin has not been reported. The objective of this research was to determine the antioxidant (phenolic) content and antioxidant capacity of peach skin from various peach varieties grown in South Carolina.

Researchers have indicated that phenolic compounds play an important role in antioxidant activity of peaches (Francisco et al., 2001). Major phenolic compounds found in peaches are caffeic acid, chlorogenic acid, leucoanthocyanins, catechins and flavonols (Francisco et al., 2001; Campbell, & Padilla-Zakour, 2013). Other antioxidants found in peaches include ascorbic acid and carotenoids. However, the content of ascorbic acid and carotenoids in peaches is relatively low (Francisco et al., 2001). Desmond & Daniele (2008) pointed out that phenolic compounds are concentrated in peach skin.

Since antioxidants act by several mechanisms and no one assay can capture the different modes of action, several different methods should be used to measure antioxidant capacity. Since 1958, numerous in vitro antioxidant assays have been

proposed including 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Kurechi, Kikugawa, & Kato, 1980), ferrous chelating capacity (FIC) (Decker, & Welch, 1990), trolox equivalent antioxidant capacity assay (TEAC)(Miller et al., 1993), oxygen radical absorbing capacity (ORAC)(Cao, Alessio, & Cutler, 1993), total radical trapping antioxidant parameter (TRAP)(Lissi et al., 1995), ferric ion reducing antioxidant power assay (FRAP) (Benzie, & Strain, 1996), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay (Re et al., 1999). All of these antioxidant assays have been applied to test the antioxidant activity in vitro. Mostly, they are used to evaluate fruits, vegetables, plant extracts, beverages and nutritional supplements. Total phenolic assay, DPPH assay and FRAP assay are the most popular methods applied in research.

2. Material and Methods

2.1 Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH), iron chloride hexahydrate ($\text{FeCl}_3 \bullet 6\text{H}_2\text{O}$), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate(ferrozine), Folin & Ciocalteu's phenol reagent, sodium carbonate, iron chloride tetrahydrate ($\text{FeCl}_2 \bullet 4\text{H}_2\text{O}$) were purchased from Sigma- Aldrich. 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma-Fluka. L-ascorbic acid, gallic acid were purchased from Sigma Life Science. Ethyl alcohol (absolute, anhydrous, ACS/USP Grade) was purchased from Pharmco-AAPER. Glacial acetic acid was purchased from BDH. Hydrochloric acid solution certified 0.1N was purchased from Fisher Scientific.

2.2 Peaches

Thirteen cultivars of peaches from South Carolina, namely Summer Gold, Contender, July Flame, Scarlet Prince, Fire Prince, Cary Mac, Ruby Prince, Red Globe, Norman, Bounty, Early August Prince, Flame Prince, O'Henry at harvest stage between July 12 and August 15, 2013, were obtained from a local farm. These varieties were chosen since these were the most popular during the peak growing season in this region of the Southeast US.

2.3 Color Analysis

Color of peach skin was measured on a model CR-400 chroma meter (Minolta CO. LTD.) by placing the colorimeter orifice directly on the peach surface prior to skin removal. Four peaches of each variety were randomly pick, four measurements on different location of each peach was evaluated by chroma meter. The color was expressed as CIE 1976 $L^*a^*b^*$, chroma (C^*), and hue (h^*), with L^* representing the lightness of the color ($L^*=0$ yields black and $L^*=100$ indicates diffuse white), a^* represents the redness to greenness of color (a^* negative values indicate green while positive values indicate magenta), b^* represents the yellowness to blueness of color (b^* negative values indicate blue and positive values indicate yellow). C^* represents the intensity or purity of color, while h^* represents hue of color.

2.4 Preparation of Peach Skin Samples

Based on the size of different peach varieties, 10 to 15 peaches of each variety were selected for sampling. Peaches were subdivided into 4 groups randomly. Peach skins were knife-peeled by hand and placed in marked sample bags and sealed. Those bags were frozen at -80°C until analyzed. For each peach variety, four antioxidant assays were performed with one bag of peach skins used for each replication. In total, four replications of each antioxidant assays were analyzed.

2.5 Extraction Method

Extraction procedures were based on methods outlined by Lim et al., (2007). For one replication, one frozen sample bag of each variety was held at room temperature for 10 mins prior to extraction. After that, the remaining flesh was scraped quickly from the peach skin and 10 g of peach skin was randomly taken from each sealed sample bag for analysis. Sample weights were recorded on a model B204-S College Monobloc analytical balance (Mettler Toledo, Toledo, OH). Moisture content was detected at the same time with a model HB43-S Mettler Toledo. Peach skins were mixed in a blender for 6 seconds with 200 ml 50% ethanol and then homogenized for 30 s. Homogenization was performed using a model PT 10/35 polytron with a model PCU 11 power control unit (Kinematica, Swizerland). The homogenized solution was placed in an model 5510R-DTH Ultrasonic unit. (Output 42KHz +/- 6%, Branson ultrasonics corporation, Danbury, CT) for 30min at room temperature and then centrifuged (Beckman Coulter Avanti J-26S XPI Centrifuge, Jersey city, NJ) at 15008 g, 5°C for 15 minutes. In total, four replications

of extraction were performed and each supernatant was recovered carefully for antioxidant activity analysis.

2.6 Antioxidant Activity Evaluation

2.6.1 Total phenolics content

Total phenolic compounds in each peach skin extract were determined with Folin–Ciocalteu reagent according to the method of Singleton et al. (1999). Gallic acid was used as a standard phenolic compound. Briefly, 0.04 ml of each peach skin extract was diluted with distilled water (3.16 ml) and 0.2 ml of Folin–Ciocalteu reagent was added and mixed thoroughly. Within 8 minutes, 0.6 ml of Na₂CO₃ (20%) was added, mixed, and incubated 30 min in a 40°C water bath. The absorbance was measured at 765 nm with a spectrophotometer (Model 4001/4 Genesys 20 Thermo Fisher Scientific, Waltham, MA). Results were expressed as gallic acid equivalent (mg gallic acid/g dried extract).

2.6.2 DPPH free radical scavenging assay

Radical scavenging activity of peach skin extract was measured according to the method of Molyneux (2004). Briefly, 0.4 ml of each peach skin extract at various concentrations was added to 2 ml of a DPPH solution (0.2 mM in 50% ethanol) and kept for 30 min at room temperature. The absorbance was measured at 517nm with a spectrophotometer (Model 4001/4 Genesys 20 spectrophotometer Thermo Fisher Scientific, Waltham, MA). L-ascorbic acid (50uM-400uM) was used as a standard. Results were expressed as ascorbic acid equivalent (mg ascorbic acid/g dried extract).

2.6.3 Ferric reducing antioxidant power (FRAP) assay

Reducing power of peach skin extract was determined by FRAP assay described by Benzie and Strains (1996). Briefly, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $\text{FeCl}_3 \bullet 6\text{H}_2\text{O}$ were freshly prepared. pH was measured using a pH meter (by model S/N 004602, Orion Research Inc., city, state) Working FRAP reagent was prepared by mixing acetate buffer, TPTZ reagent and $\text{FeCl}_3 \bullet 6\text{H}_2\text{O}$ in the ratio of 10:1:1 at the time of use. 100ul of each peach skin extract was mixed with 3ml of working FRAP reagent and then kept at 37°C water bath for 4 minutes. The absorbance was measured at 593nm with a spectrophotometer (model 4001/4 Genesys 20 spectrophotometer, Thermo fisher scientific, Waltham, MA). Ascorbic acid (100uM - 1000 uM) was used as a standard. The reducing power was expressed as ascorbic acid equivalent (mg Ascorbic Acid/ g dried extract).

2.6.4 Ferrous ion chelating (FIC) assay

Ferrous ion-chelating potential of peach skin extract was determined according to the method of Gulcin et al. (2008). Briefly, 1 ml of peach skin extract was mixed with 0.2 ml of 2 mM FeCl_2 . Then 2.4 ml 50% ethanol was added to the mixture. The reaction was initiated by the addition of 0.4 ml of 5mM ferrozine. The mixture was allowed to sit at room temperature for 10 minutes before absorbance was measured at 562nm with a spectrophotometer (Model 4001/4 Genesys 20 spectrophotometer Thermo fisher

scientific, Waltham, MA). EDTA was used as positive control. The ability of the extract to chelate ferrous ion was calculated using the following equation

$$\text{Chelating ability (\%/mg/ml)} = [(A_0 - A_1)/A_0] \times 100\% / \text{Conc1},$$

where A_0 is the absorbance of the control, A_1 is the absorbance of peach skin sample or EDTA, Conc1 is the concentration of peach skin extracts.

2.7 Statistical Analysis

Statistical analysis was carried out in SAS 9.3 software (released in July 2011). An ANOVA table was generated to determine if peach varieties affected each assay, then when variety had a significant effect ($p < 0.05$), the Tukey post hoc test was used to separate means ($p < 0.05$) among the 13 peach varieties. Correlation of the four antioxidant assays was also performed using Pearson Correlation.

3. Results and Discussion

3.1 Color Analysis

Lightness and hue values varied among the 13 varieties evaluated with Cary Mac, Ruby Prince, Flame Prince being significantly lighter than all other varieties except for Contender (Figure 6). Also, the lightness of Norman was significantly lower than other peaches excluding July Flame and Summer Gold. The Norman variety was significantly lower from other peaches in chroma while other peaches had similar color purity (Figure 7). For hue value, Cary Mac and Flame Prince were also significantly higher than all

other peach varieties except Ruby Prince and Contender (Figure 8). In summary, Norman, Cary Mac, Ruby Prince and Flame Prince differed in several color parameters compared to other peach varieties (Table 1).

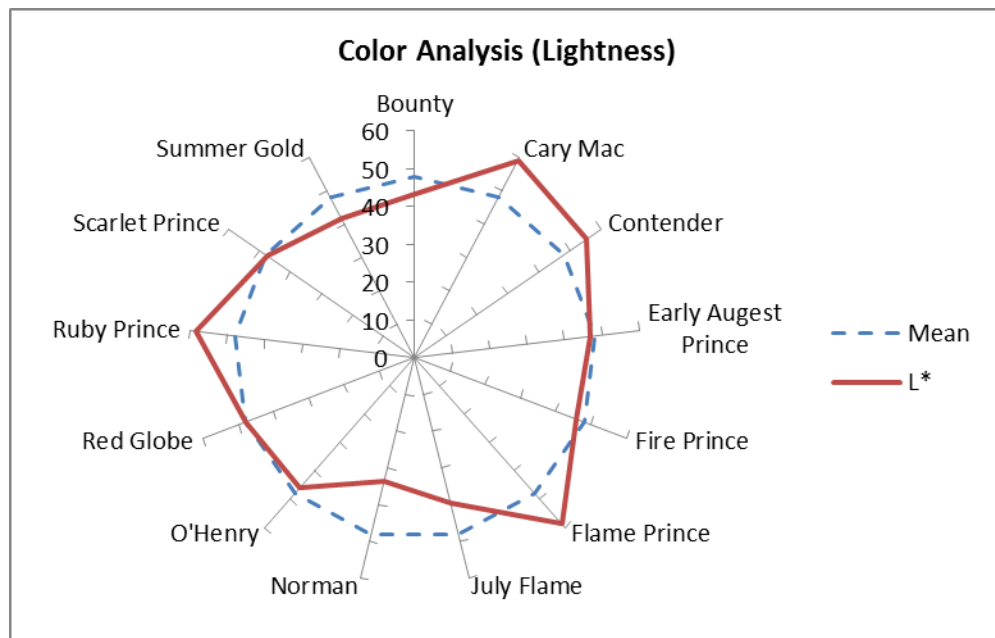


Figure 6. Color Analysis (L*) of 13 cultivars of peaches

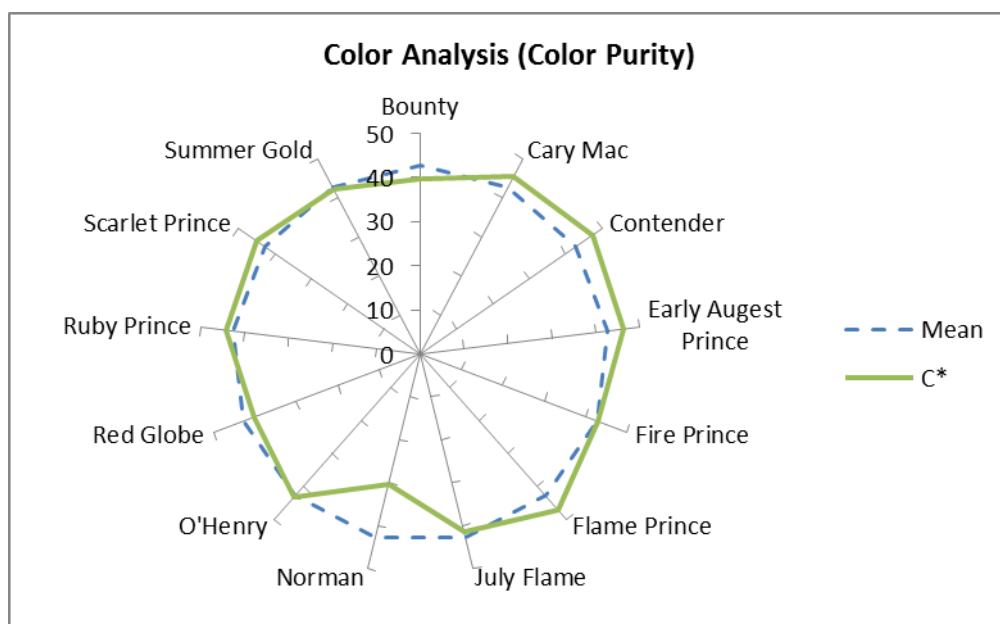


Figure 7. Color Analysis (C*) of 13 cultivars of peaches

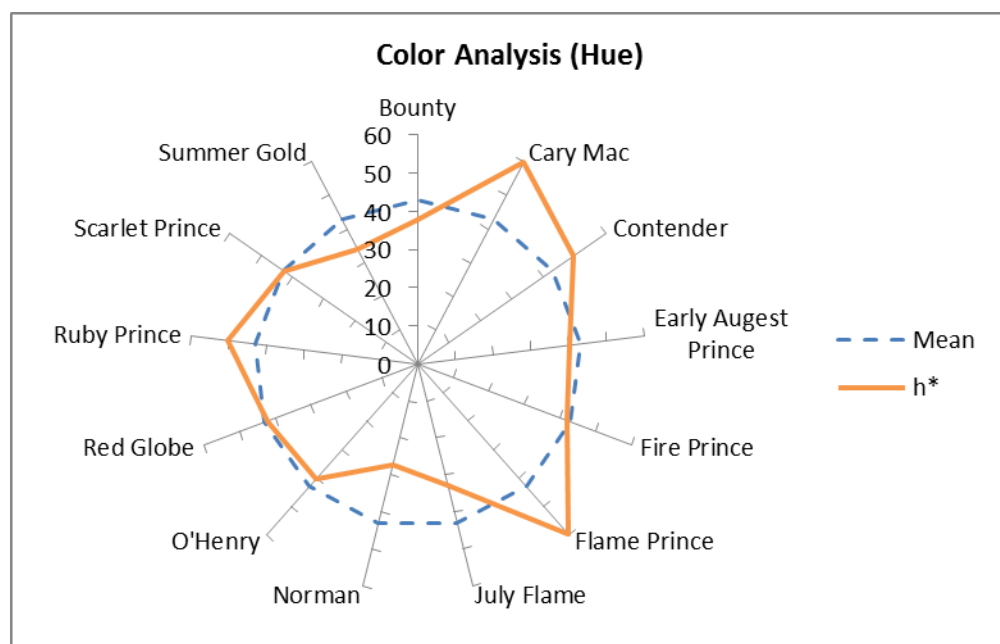


Figure 8. Color Analysis (Hue) of 13 varieties of peaches

Table 1. Color analysis of 13 cultivars of peach skins

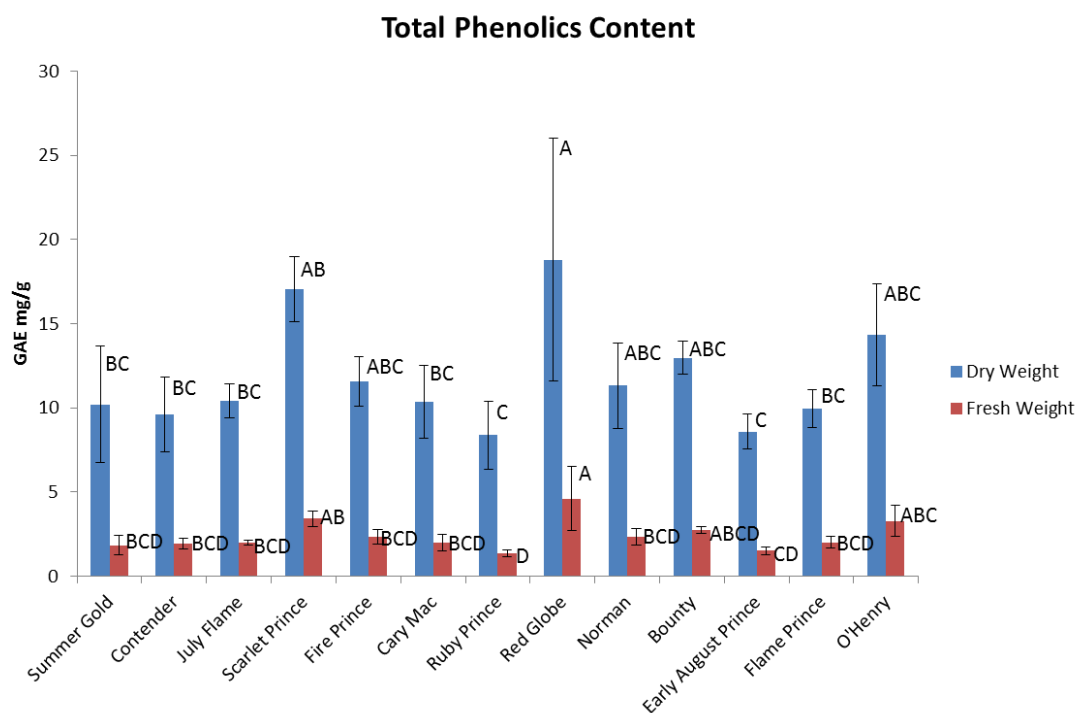
Color	L*		C*		h*		a*		b*	
Cultivar	Estimate	Standard	Estimate	Standard	Estimate	Standard	Estimate	Standard	Estimate	Standard
	Means	Deviation	Means	Deviation	Means	Deviation	Means	Deviation	Means	Deviation
Bounty	43.32 ^C	7.06	39.69 ^B	7.72	37.76 ^{BCD}	12.03	30.24 ^{ABC}	6.40	24.48 ^{DEF}	9.12
Cary Mac	59.07 ^A	4.97	45.49 ^{AB}	2.16	59.82 ^A	11.14	22.48 ^D	7.74	38.61 ^{AB}	4.77
Contender	55.30 ^{AB}	6.40	47.52 ^A	3.40	49.72 ^{AB}	9.66	30.08 ^{ABC}	5.19	36.00 ^{ABC}	6.82
Early August Prince	47.03 ^{BC}	4.22	46.27 ^{AB}	3.75	40.37 ^{BCD}	4.66	35.05 ^A	2.80	30.00 ^{ABCDE}	4.41
Fire Prince	45.98 ^{BC}	9.19	42.92 ^{AB}	5.76	41.93 ^{BC}	13.69	30.43 ^{ABC}	5.58	28.62 ^{BCDE}	10.28
Flame Prince	58.83 ^A	8.34	46.92 ^{AB}	3.98	59.58 ^A	13.14	22.59 ^D	6.75	39.92 ^A	8.62
July Flame	39.73 ^{CD}	4.23	41.47 ^{AB}	5.39	32.98 ^{CD}	5.74	34.45 ^{AB}	3.66	22.73 ^{EF}	5.76
Norman	33.56 ^D	5.77	30.26 ^C	8.82	27.11 ^D	6.44	26.41 ^{CD}	6.61	14.43 ^F	6.69
O'Henry	45.89 ^{BC}	8.72	43.33 ^{AB}	4.83	40.18 ^{BCD}	11.50	32.04 ^{ABC}	3.99	27.95 ^{CDE}	9.03
Red Globe	47.73 ^{BC}	13.72	40.33 ^{AB}	9.69	42.36 ^{BC}	19.58	26.39 ^{CD}	6.68	27.62 ^{CDE}	15.07
Ruby Prince	58.30 ^A	7.38	44.31 ^{AB}	2.83	50.27 ^{AB}	11.05	27.63 ^{BCD}	5.94	33.67 ^{ABCD}	6.56
Scarlet Prince	47.48 ^{BC}	8.13	45.10 ^{AB}	6.61	42.55 ^{BC}	11.52	32.11 ^{ABC}	5.78	30.59 ^{ABCDE}	9.05
Summer Gold	41.81 ^{CD}	7.09	42.10 ^{AB}	7.40	34.05 ^{CD}	8.55	34.03 ^{AB}	4.35	24.10 ^{DEF}	8.45

A-E means within the same column with the same subscript are not significantly different (P>0.05)

3.2 Total Phenolics Content

Gallic acid was used as the standard for phenolic content therefore experimental results were expressed as gallic acid equivalents (GAE) in both dry weight and fresh weight.

Phenolic content differed across the varieties of peaches tested. For dry weight analysis, among all the varieties, Red Globe had the highest mean value of phenolic compounds while Ruby Prince had the lowest mean value of phenolic compound (Figure 9). The order from greatest mean to lowest mean of total phenolics content was Red Globe, Scarlet Prince, O'Henry, Bounty, Fire Prince, Norman, July Flame, Cary Mac, Summer Gold, Flame Prince, Contender, Early August Prince, Ruby Prince. Red Globe had a significantly greater ($p < 0.05$) phenolic content than July Flame, Cary Mac, Summer Gold, Flame Prince, Contender, Early August Prince and Ruby Prince while the Scarlet Prince variety had greater ($p < 0.05$) phenolic content than Early August Prince and Ruby Prince. Red Globe and Summer Gold varieties had a large variation while the Scarlet Prince variety had both a high phenolic content and a relatively small range of variation in phenolic content (Figure 10).



A-D means within the same color bar having the same subscript are not significantly different ($P > 0.05$)

Figure 9. Antioxidant activity of Total Phenolics assay

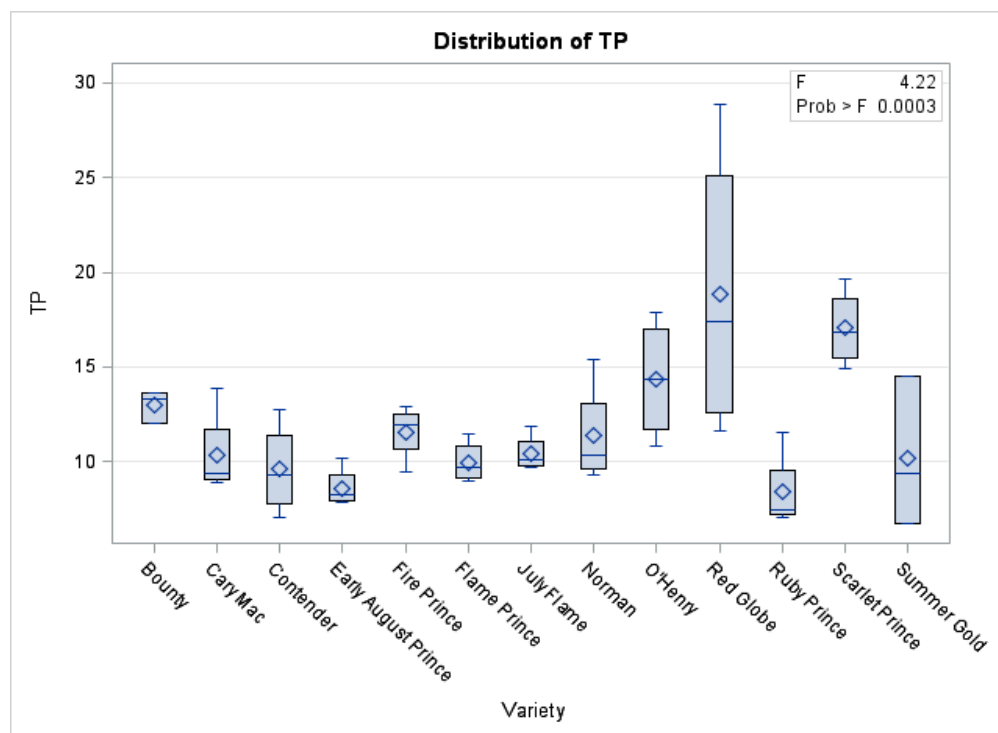
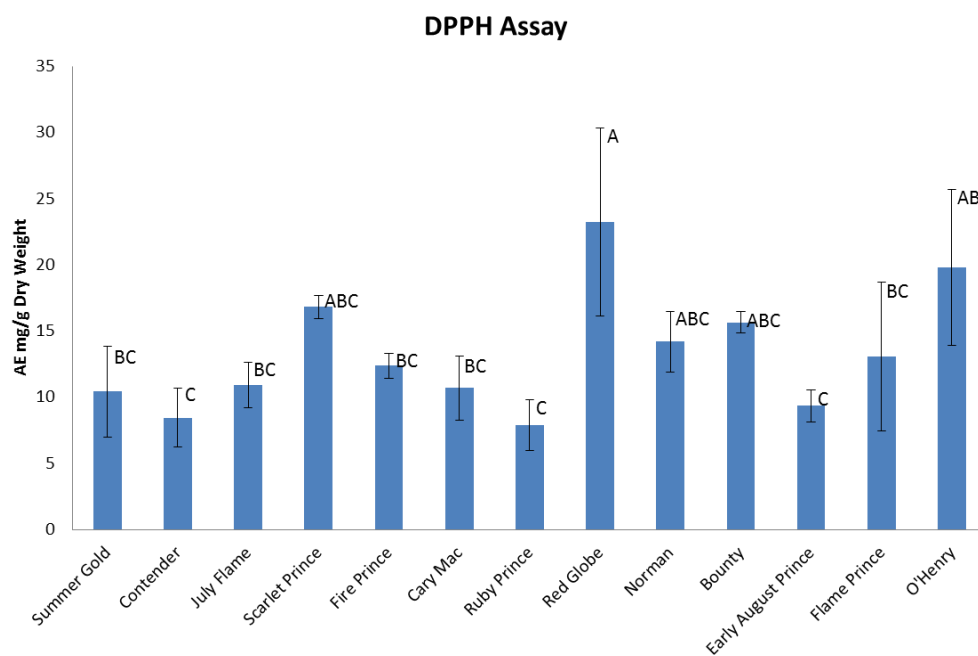


Figure 10. Box and whisker of Total Phenolics assay (Dry Weight)

3.3 DPPH Assay

Ascorbic acid was used the standard for the DPPH assay thus results were expressed as ascorbic acid equivalent (AE). The Red Globe variety had the highest mean value of radical scavenging activity (Figure 11). The order from highest mean value to lowest mean value of radical scavenging activity was Red Globe, O'Henry, Scarlet Prince, Bounty, Norman, Flame Prince, Fire Prince, July Flame, Cary Mac, Summer Gold, Contender, Early August Prince, Ruby Prince. Red Globe peaches were not significantly different ($p>0.05$) from O'Henry, Scarlet Prince, Bounty and Norman but had greater ($p<0.05$) radical scavenging activity than other varieties. O'Henry had greater ($p<0.05$) scavenging activity than Early August Prince, Contender and Ruby Prince. Box and whisker plot revealed that Red Globe and O'Henry peach samples had more variation than others and that Scarlet Prince (as with phenolic content) had low variation and relatively high scavenging activity (Figure 12).



A-C means with the same subscript are not significantly different ($P > 0.05$).

Figure 11. Antioxidant activity of DPPH assay

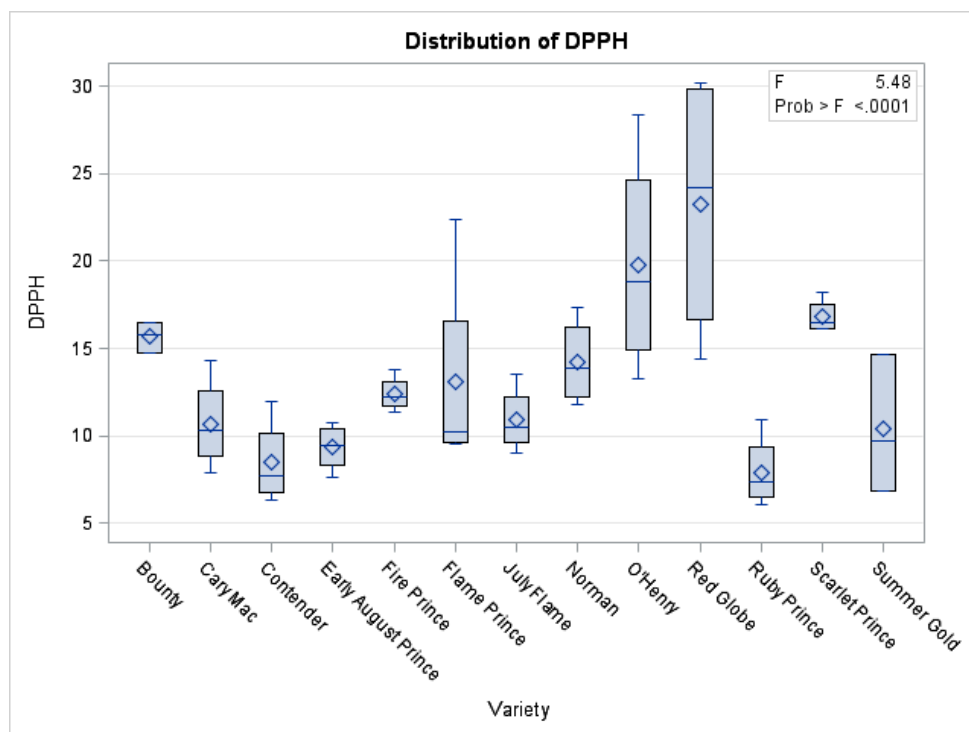
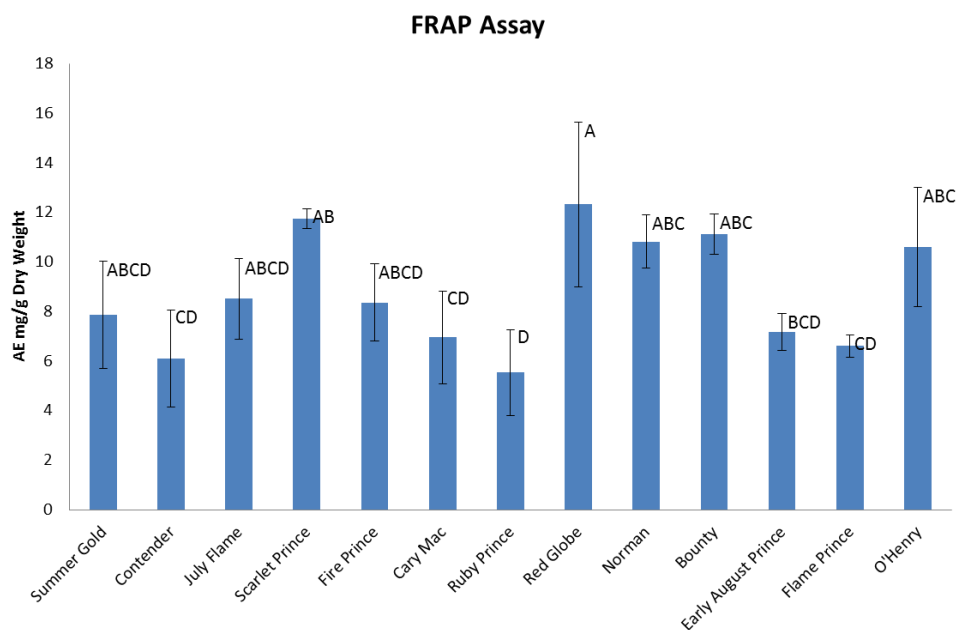


Figure 12. Box and whisker plot of DPPH assay

3.4 FRAP Assay

Ascorbic acid was used as the standard reference therefore results were expressed as ascorbic acid equivalents (AE). The order from highest mean value to least mean value of reducing ability of peach varieties was Red Globe, Scarlet Prince, Bounty, Norman, O’Henry, July Flame, Fire Prince, Summer Gold, Early August Prince, Cary Mac, Flame Prince, Contender, Ruby Prince (Figure 13). Red globe had significantly more ($p<0.05$) reducing ability than Early August Prince, Cary Mac, Flame Prince, Contender and Ruby Prince. Ruby Prince was lower ($p<0.05$) in FRAP AE than O’ Henry, Norman, Bounty, Scarlet Prince and Red Globe. Box whisker distribution of FRAP assay showed that the Red Globe peach variety had the most variation (Figure 14).



A-D means with the same subscript are not significantly different ($P>0.05$).

Figure 13. Antioxidant activity of FRAP assay

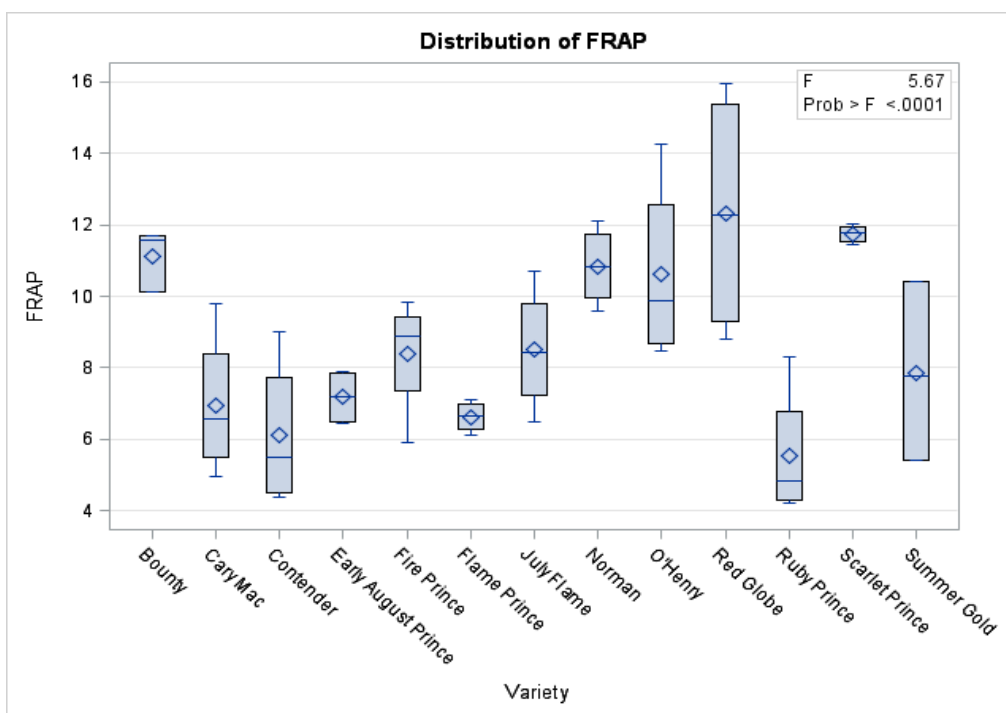
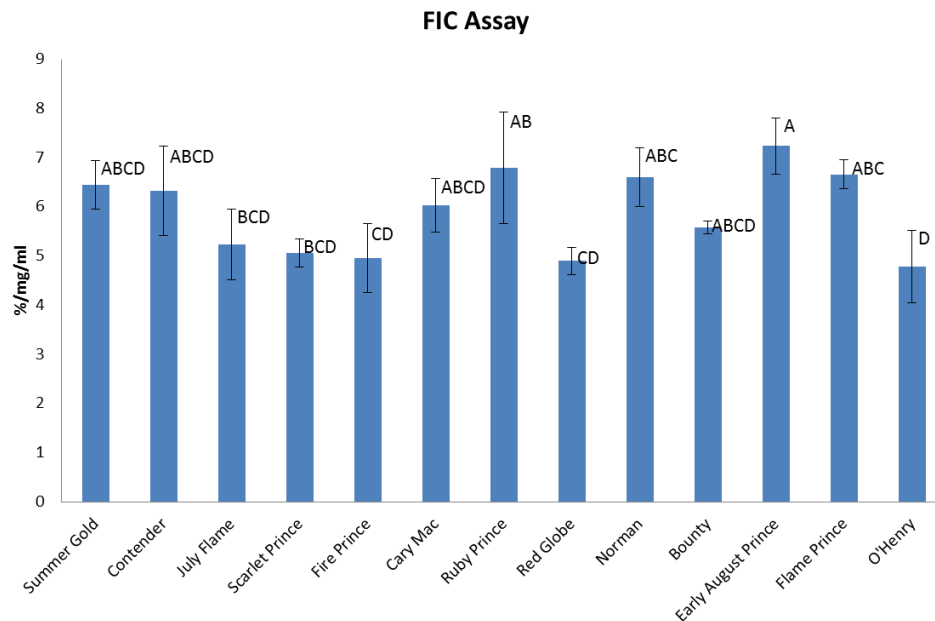


Figure 14. Box and whisker plot of FRAP assay

3.5 FIC Assay

Compared with other antioxidant measurements, results of Ferrous Ion Chelating assay differed which may be due to the fact that FIC measures chelating ability, a different mode of antioxidant action compared to the other assays used. The order of highest mean value to lowest mean value of chelating ability was Early August Prince, Ruby Prince, Norman, Flame Prince, Summer Gold, Contender, Cary Mac, Bounty, July Flame, Scarlet Prince, Fire Prince, Red Globe, O'Henry (Figure 15). Early August Prince was significantly greater ($p < 0.05$) than July Flame, Scarlet Prince, Fire Prince, Red Globe and O'Henry. O'Henry had significantly lower ($p < 0.05$) chelating ability than Early August Prince, Ruby Prince, Flame Prince and Norman. For chelating ability, Ruby Prince

peaches had the largest variation while Bounty, Flame Prince, Red Globe and Scarlet Prince varied the least (Figure 16).



A-D means with the same subscript are not significantly different ($P > 0.05$)

Figure 15. Antioxidant activity of FIC assay

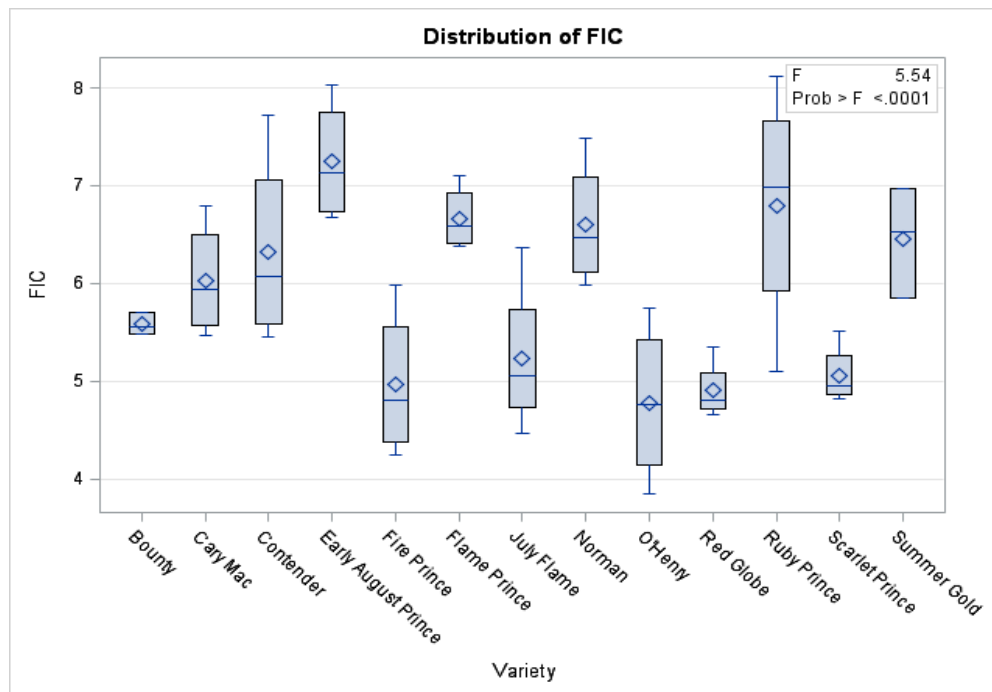


Figure 16. Box and whisker plot of FIC assay

3.6 Correlation of four antioxidant assays

There is no one assay that measures all aspects of antioxidant capacity. Therefore, antioxidant assays based on different modes of action were performed to evaluate overall antioxidant capacity. Correlation of different antioxidant assays was performed to verify the relationship between assays in evaluating different peach varieties. P value of all the Pearson Correlation less than 0.05, the correlation is statistically significant. Total phenolic assay and DPPH assays were the most closely correlated with an R value of 0.92.

Table 2. Correlation of four antioxidant assays

Pearson Correlation Coefficients (R), N = 13				
Prob > r under H0: Rho=0				
		DPPH Assay	FRAP Assay	FIC Assay
TP Assay	R	0.92	0.88	-0.76
	P	<.0001	<.0001	0.0024
DPPH Assay	R		0.87	-0.69
	P		<.0001	0.0084
FRAP Assay	R			-0.66
	P			0.014

3.7 Correlation of color and total phenolic content

Correlation of color and total phenolic content was performed to test the relationship of color and phenolic content. P value of all Pearson correlation higher than 0.05, which means correlations were not statistically significant. Therefore, Pearson correlation coefficients indicated that the peach skin color measurements were not significantly correlated to total phenolic content of peaches.

Table 3. Correlation of color and total phenolic content

		Pearson Correlation Coefficients (R), N = 13				
		Prob > r under H0: Rho=0				
Total Phenolics		L*	a*	b*	C*	h*
	R	-0.2365	-0.0212	-0.2045	-0.2234	-0.19
	P	0.4365	0.9451	0.5027	0.4632	0.5341

4. Conclusion

The total phenolic content and antioxidant activity of peach skin were affected by peach cultivar. The range of total phenolic content was 8.38 – 18.81 (Gallic Acid Equivalent mg/g dry weight). Babbar et. al. (2011) reported total phenolics content of six fruit residues which are kinnow seed 3.68 mg GAE/g dry weight, kinnow peel 17.5 mg GAE/g dry weight, litchi seed 17.9 mg GAE/g dry weight, litchi pericarp 24.6 mg GAE/g dry weight, grape seed 37.4 mg GAE/g dry weight, banana peel 3.8 mg GAE/g dry weight. Though the total phenolic content of peach skins were lower than grape seed, all the

peach skins from different varieties were higher than kinnow seed and banana peel. Some varieties like Red Globe and Scarlet prince were similar to kinnow peel and litchi seed. In general, peach skin possessed good antioxidant capacity. The order of antioxidant strength of the TP, DPPH and FRAP assays followed a similar trend due to cultivar while the results of FIC assay did not match the other assays.

Previous research has showed that total phenolic content of O'Henry peach skin evaluated by HPLC-DAD method was 120.2mg/100g fresh weight (Maria et al., 2002) In the current study, total phenolic content (Gallic acid equivalent) of O'Henry was 328.7mg/100g fresh weight. Since total phenolic content was expressed directly in previous research but expressed indirectly as Gallic acid equivalent in current research, they cannot be compared directly. Factors which may influence the total phenolic contents of peaches included growing environment, weather conditions, timing of harvest and soil conditions.

Correlations among TP, DPPH and FRAP assays were all high while the correlation coefficient of FIC assay to the other assays was not as high. This is case since the FIC assay measures chelating ability while the other assays measure phenolics and reducing or radical scavenging which are closely related to phenolic structure. While both DPPH and FRAP measure reducing reactions, the antioxidant capacity evaluated of DPPH assay was generally higher than FRAP assay. DPPH assay is based on the transfer of a hydrogen atom, while FRAP assay depends on electron transfer, particularly the

reduction of Fe(III). Not all the antioxidants can reduce the Fe(III), and this could be the reason the FRAP assay gave a lower value than the DPPH assay. Correlation of peach skin color with phenolic content showed that color could not indicate the phenolic content of different varieties of peaches.

Future studies may include HPLC analysis of peach skin components and antioxidant activity tests in vivo. Also, since peach skin extract has good antioxidant activity, finding a way to produce a natural additive for other food products could be an economic benefit to the peach industry.

References

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, 44, 391-396.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem.* 239, 70-76.

Block, G., Patterson, B., & Subar, A. (1992). Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer.* 18, 1–29.

Brunke, H., Chang, M., Huntrods, D., & McKee G. 2013. peach profile. Agriculture Marketing Resource Center (AgMRC), http://www.agmrc.org/commodities__products/fruits/peach-profile/

Campbell, O.E., & Padilla-Zakour, O.I. (2013). Phenolic and carotenoid composition of canned peaches (*Prunus persica*) and apricots (*Prunus armeniaca*) as affected by variety and peeling. *Food Research International*, 54, 448-455.

Cao, G., Alessio, H.M., & Cutler, R.G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine.* 14(3), 303-311.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* 38, 674–677.

Desmond, R. L., & Daniele, B. (2008) The peach botany, production and uses. CAB international. In Crisosto, C.H., & Valero D, *Harvesting and postharvest handling of peaches for the fresh market* (pp. 575-594). CAB International.

- Francisco, A. T., María, I. G., Paedar, C., Andrew, L. W., Betty, H.P., & Adel A. K. (2001). HPLC–DAD–ESIMS Analysis of Phenolic Compounds in Nectarines, Peaches, and Plums. *J. Agric. Food Chem.* 49 (10), 4748–4760.
- Gulcin, I., Tel, A.Z., & Kirecci, E. (2008). Antioxidant, Antimicrobial, Antifungal, and Antiradical Activities of *Cyclotrichium niveum* (boiss.) Manden and Scheng. *International Journal of Food Properties.* 11, 450-471.
- Kurechi, T., Kikugawa, K., & Kato, T. (1980). Studies on the Antioxidants Hydrogen Donating Capability of Antioxidants to 2,2-Diphenyl-1-picrylhydrazyl. *Chem. Pharm. Bull.* 28(7), 2089-2093.
- Lim, Y.Y., Lim, T.T., & J.J. Tee. (2007). Antioxidant Properties of Several Tropical Fruits: A Comparative Study. *Food Chem.* 103, 1003-1008.
- Lissi, E., Salim-Hanna, M., Pascual, C., & del Castillo, M.D. (1995). Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. *Free Radical Biology and Medicine.* 18(2), 153-158.
- Maria, I. G., Francisco, A. T., Betty, H., & Adel A. K. (2002). Antioxidant Capacities, Phenolic Compounds, Carotenoids, and Vitamin C Contents of Nectarine, Peach, and Pulm Cultivars from California. *J. of Agric. and Food Chem.* 50, 4976-4982.
- Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.* 26(2), 211-219.

Miller, N.J., Rice-Evans, C.A., Davies, M.J., Gopinathan, V., & Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates. *Clin. Sci.* 84, 407-412.

Ness, A.R., & Powles, J.W. (1997). Fruits and vegetables and cardiovascular disease: a review. *Int. J. Epidemiol.* 26, 1–13.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* 26(9/10), 1231-1237.

Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin- Ciocalteu Reagent. *Methods in Enzymology.* 299, 152-178.

USDA. (2013). Noncitrus fruits and nuts 2012 preliminary summary. pp 58.

http://www.nass.usda.gov/Publications/Todays_Reports/reports/ncit0113.pdf.

APPENDICES

Appendix A: 13 Varieties of Peach Extracts

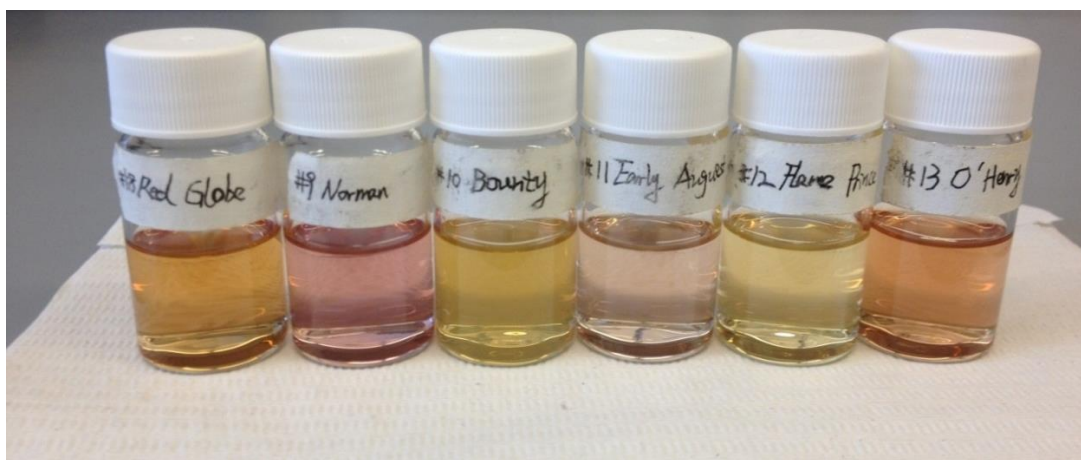


Figure A-1: 13 varieties of peach skin extracts

Appendix B: 13 Varieties of Peach Samples

#1 Summer gold



#2 Contender



#3 July Flame



#4 Scarlet Prince



#5 Fire Prince



#6 Cary Mac



#7 Ruby Prince



#8 Red Globe



#9 Norman



#10 Bounty



#11 Early August Prince



#12 Flame Prince



#13 O'Henry



Figure B-1: 13 Varieties of Peach Samples

Appendix C: Description of 13 cultivars of peaches

Table C- 1 Description of 13 varieties of peaches

Cultivar	Type	Flesh	Harvest time	Description
Summer Gold	Clingstone	Yellow	Mid July	A large size, globose peach. Flesh firm with good flavor and eating quality. A high degree of attractive red skin color.
Contender	Freestone	Yellow	Early to Mid-July	A medium large to large sized, globose peach. 70-90% crimson red over a scarlet yellow ground color. The flesh is firm with very good flavor and resistant to browning.
July Flame	Freestone	Yellow	Early to Mid-July	A brilliant and complete bright red, very firm peach. A beautiful scarlet orange skin color over 90 % of the surface.
Scarlet Prince	Freestone	Yellow	Early to Mid-August	A medium-large, globose peach. 80-90% scarlet red over greenish yellow ground color. The flesh is firm to very firm with very good flavor
Fire Prince	Freestone	Yellow	Early July	A medium-large, globose peach.
Cary Mac	Freestone	Yellow	Late June	A medium to large peach, fairly uniform, skin yellow with red cheek, flesh yellow with tendency of non-browning, subacid with slight soluble tannin, excellent eating quality.
Ruby Prince	Clingstone	Yellow	Mid June	A medium-large, globose, attractive peach. 80-90% scarlet

				red over yellow ground color. The flesh is moderately firm, melting flesh with good flavor.
Red Globe	Freestone	Yellow	Late June to Early July	A large and round peach with red blush over golden yellow background. It is yellow fleshed and has excellent flavor.
Norman	Freestone	Yellow	Mid July	A medium peach, round with very short and light pubescence, excellent flesh firmness and flavor. A very dark red overcolor covers 80% to 90% of the skin surface. The fruit are very resistant to flesh browning.
Bounty	Freestone	Yellow	Mid July	A large to very large, globose peach. 40-70% crimson red over light greenish-yellow ground color. The flesh is firm with very good flavor.
Early August Prince	Freestone	Yellow	Early to Mid-August	A large globose peach. 60-90% scarlet red over a yellowish red ground color. The flesh is firm, melting with very good acidic flavor.
Flame Prince	Freestone	Yellow	Late July	A medium-large, firm peach. 50-70% crimson red over a yellow ground color. The flesh is firm to very firm with very good flavor.
O'Henry	Freestone	Yellow	Early August	Bright Colored, large peach with light fuzz, streaked with red. Very firm flesh with high sugar content. Great canning and fresh eating peach.

Appendix D: Antioxidant activity of 13 varieties of peach skins

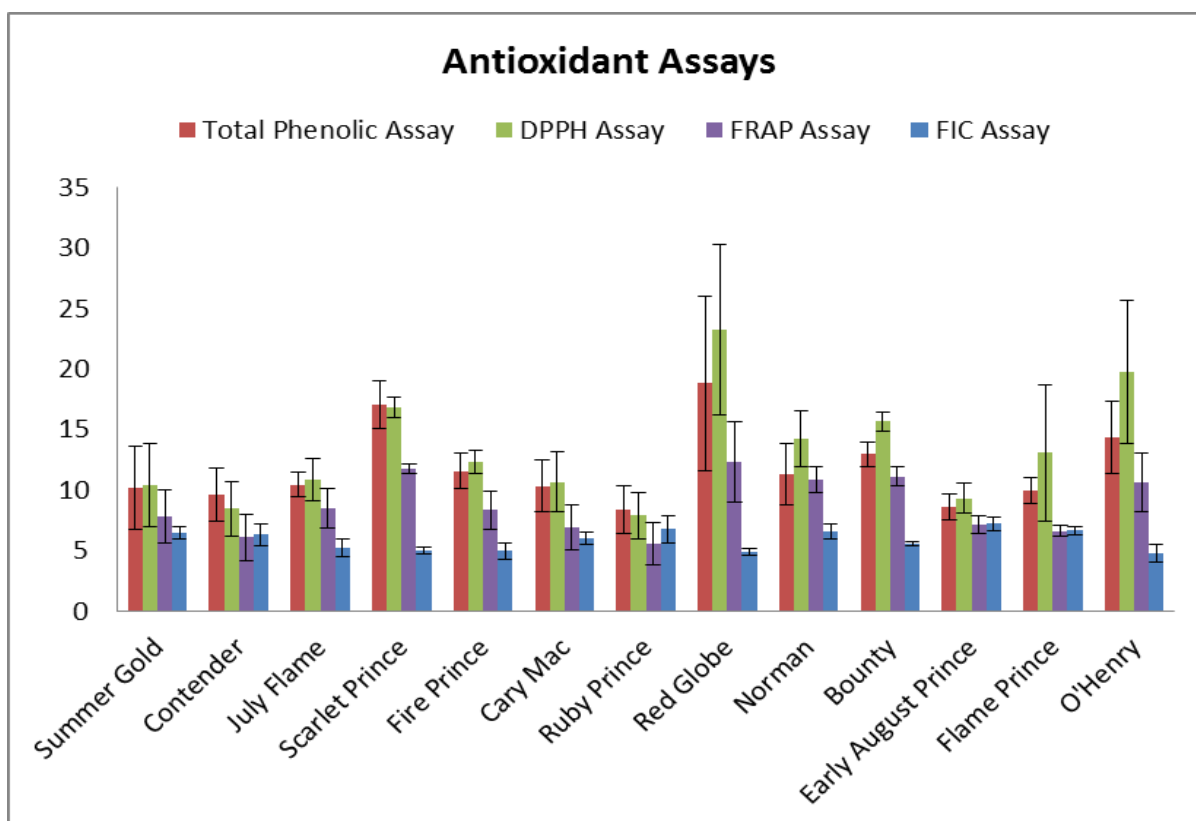


Figure D- 1 Antioxidant activity of 13 varieties of peach skins

Table D- 1 Antioxidant activity of 13 varieties of peach skins

Cultivar	Total Phenolics				DPPH Assay		FRAP Assay		FIC Assay	
	Estimate Mean	Standard Deviation	Estimate Mean	Standard Deviation	Estimate Mean	Standard Deviation	Estimate Mean	Standard Deviation	Estimate Mean	Standard Deviation
	(GAE mg/g Dry Weight)		(GAE mg/g Fresh Weight)		(AE mg/g Dry Weight)		(AE mg/g Dry Weight)		(%/concentration = %/mg/mL)	
Summer Gold	10.19 ^{BC}	3.46	1.84 ^{BCD}	0.59	10.42 ^{BC}	3.44	7.87 ^{ABCD}	2.18	6.45 ^{ABCD}	0.49
Contender	9.60 ^{BC}	2.22	1.94 ^{BCD}	0.33	8.46 ^C	2.25	6.10 ^{CD}	1.95	6.32 ^{ABCD}	0.91
July Flame	10.43 ^{BC}	1.02	1.99 ^{BCD}	0.16	10.91 ^{BC}	1.74	8.51 ^{ABCD}	1.62	5.23 ^{BCD}	0.72
Scarlet Prince	17.04 ^{AB}	1.93	3.41 ^{AB}	0.48	16.83 ^{ABC}	0.88	11.75 ^{AB}	0.41	5.06 ^{BCD}	0.28
Fire Prince	11.57 ^{ABC}	1.45	2.32 ^{BCD}	0.42	12.38 ^{BC}	0.96	8.37 ^{ABCD}	1.57	4.96 ^{CD}	0.70
Cary Mac	10.35 ^{BC}	2.16	2.01 ^{BCD}	0.49	10.69 ^{BC}	2.44	6.96 ^{CD}	1.87	6.03 ^{ABCD}	0.54
Ruby Prince	8.37 ^C	2.00	1.35 ^D	0.22	7.92 ^C	1.92	5.54 ^D	1.73	6.79 ^{AB}	1.14
Red Globe	18.81 ^A	7.21	4.61 ^A	1.90	23.25 ^A	7.08	12.33 ^A	3.32	4.90 ^{CD}	0.28
Norman	11.33 ^{ABC}	2.54	2.34 ^{BCD}	0.51	14.21 ^{ABC}	2.29	10.83 ^{ABC}	1.08	6.60 ^{ABC}	0.60
Bounty	12.95 ^{ABC}	0.98	2.76 ^{ABCD}	0.20	15.66 ^{ABC}	0.82	11.13 ^{ABC}	0.82	5.58 ^{ABCD}	0.13
Early August Prince	8.60 ^C	1.04	1.50 ^{CD}	0.23	9.34 ^C	1.21	7.17 ^{BCD}	0.74	7.24 ^A	0.57
Flame Prince	9.95 ^{BC}	1.10	2.02 ^{BCD}	0.34	13.09 ^{BC}	5.64	6.62 ^{CD}	0.45	6.66 ^{ABC}	0.30
O'Henry	14.34 ^{ABC}	3.02	3.28 ^{ABC}	0.91	19.79 ^{AB}	5.89	10.61 ^{ABC}	2.40	4.78 ^D	0.74

A-D means within the same column, the same subscript are not significantly different (P>0.05)

Appendix E: Correlation of four antioxidant assays

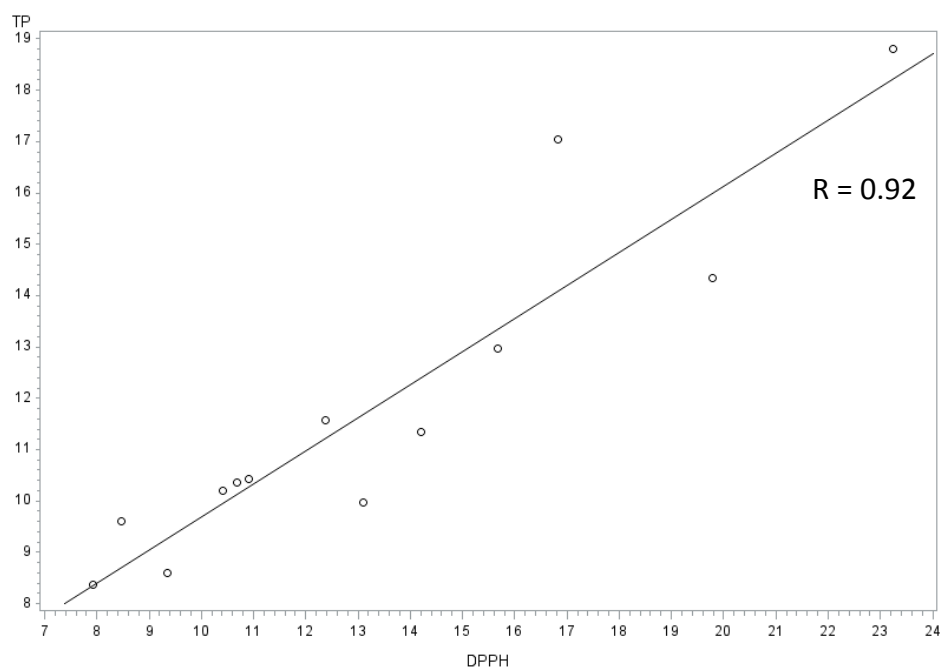


Figure E- 1 Correlation between Total Phenolics assay and DPPH assay

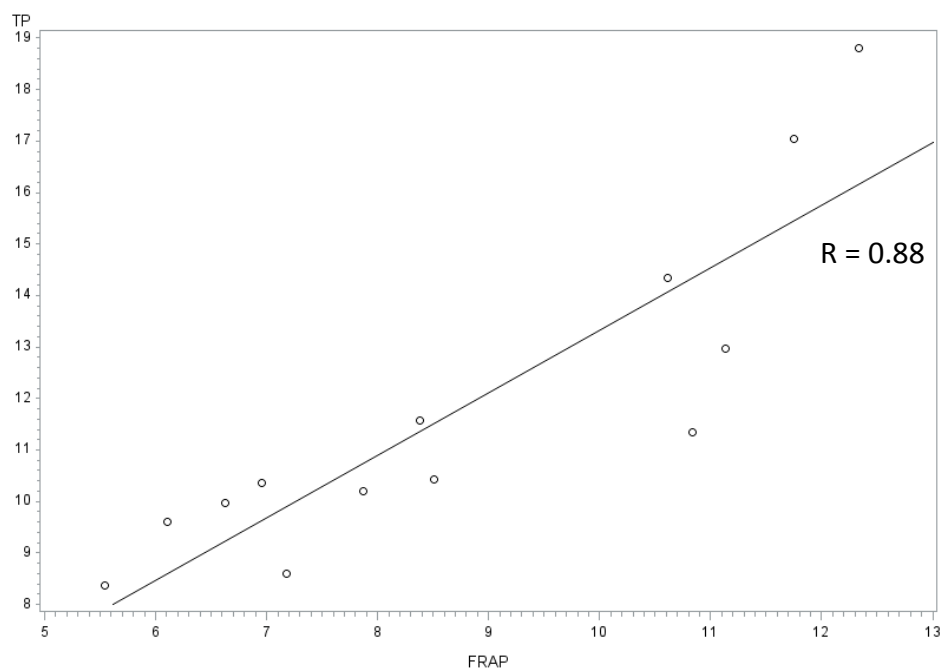


Figure E- 2 Correlation between Total Phenolics assay and FRAP assay

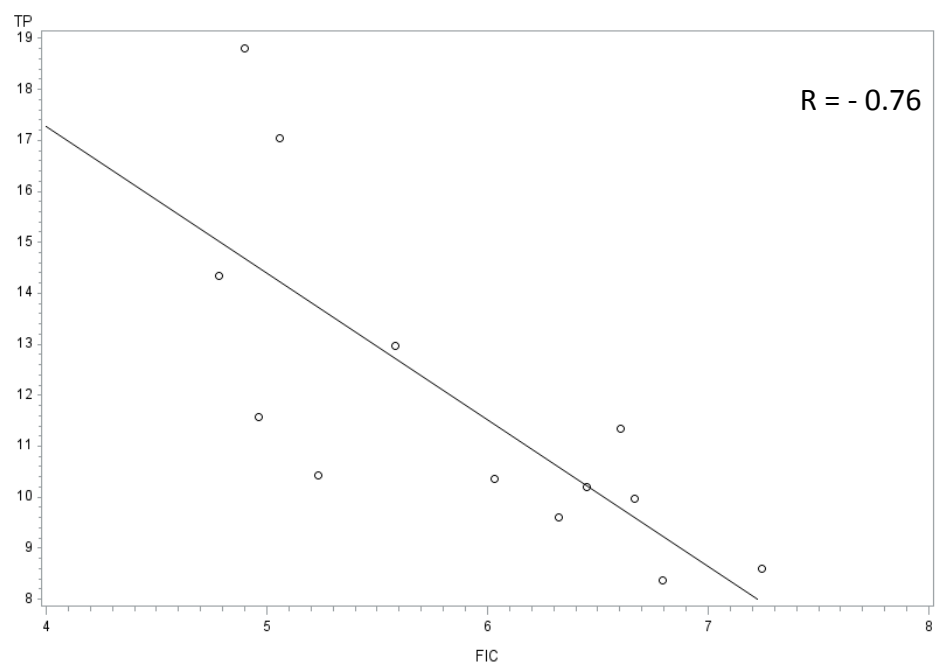


Figure E- 3 Correlation between Total Phenolics assay and FIC assay

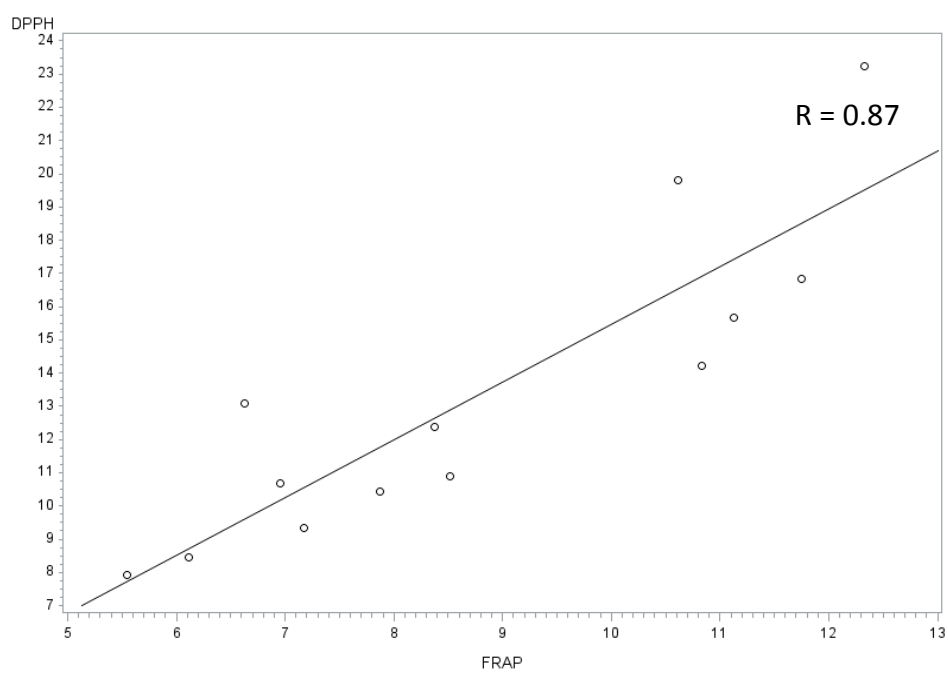


Figure E- 4 Correlation between DPPH assay and FRAP assay

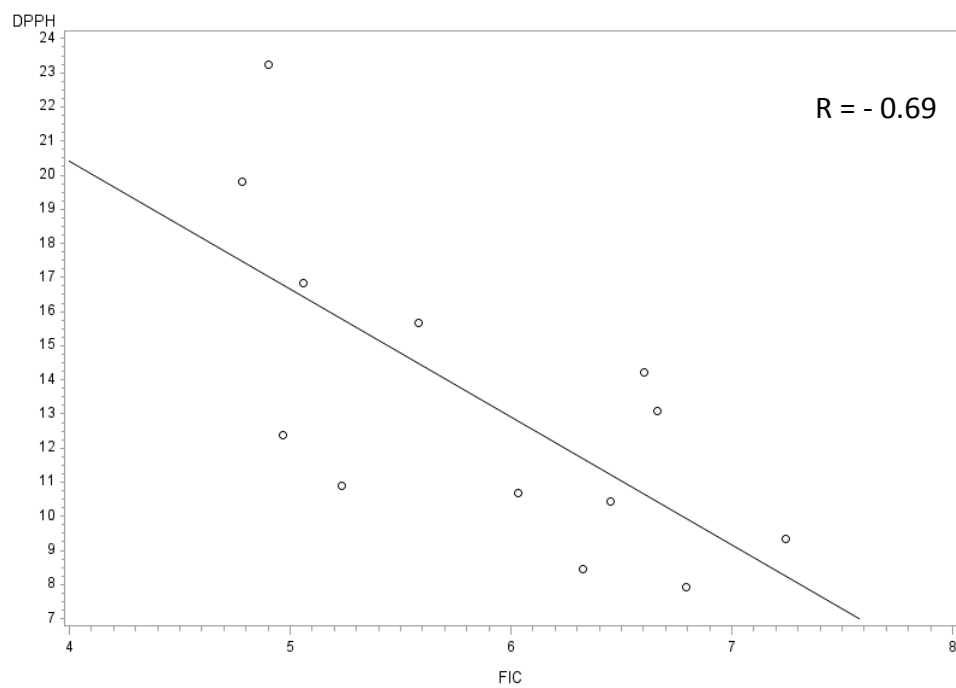


Figure E- 5 Correlation between DPPH assay and FIC assay

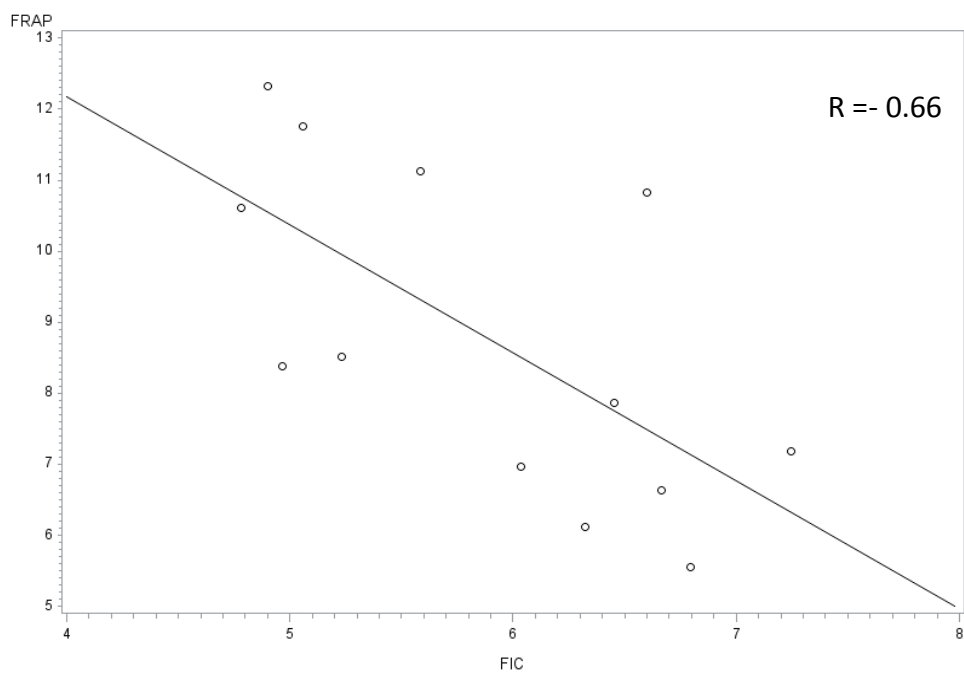


Figure E- 6 Correlation between FRAP assay and FIC assay